

TOXICOLOGICAL ABUSE PROFILE OF NEW RECREATIONAL DRUGS IN DRIVING-UNDER-THE-INFLUENCE AND POST-MORTEM CASES IN FINLAND

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LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following five publications that are referred to in the text by Roman numerals I – V:

1. Kriikku P, Wilhelm L, Schwarz O, Rintatalo J. New designer drug of abuse: 3, 4-Methylenedioxypyrovalerone (MDPV). Findings from apprehended drivers in Finland. *Forensic Sci Int* 2011; 210:195–200.
2. Kriikku P, Wilhelm L, Rintatalo J, Hurme J, Kramer J, Ojanperä I. Phenazepam abuse in Finland: Findings from apprehended drivers, post-mortem cases and police confiscations. *Forensic Sci Int* 2012; 220:111–117.
3. Kriikku P, Wilhelm L, Rintatalo J, Hurme J, Kramer J, Ojanperä I. Prevalence and blood concentrations of desoxypipradrol (2-DPMP) in drivers suspected of driving under the influence of drugs and in post-mortem cases. *Forensic Sci Int* 2013; 226:146–151.
4. Kriikku P, Rintatalo J, Wilhelm L, Hurme J, Kramer J, Ojanperä I. Pregabalin serum levels in apprehended drivers. *Forensic Sci Int* 2014; 243:112–116.
5. Kriikku P, Rintatalo J, Pihlainen K, Hurme J, Ojanperä I. The effect of banning MDPV on the incidence of MDPV-positive findings among users of illegal drugs and on court decisions in traffic cases in Finland. *Int J Legal Med* 2015; 129:741–749.

New and previously unpublished results by the author presented in this thesis are referred to by the Roman numeral VI.

ABBREVIATIONS

α -PVP	<i>alpha</i> -pyrrolidinopentiophenone
AM-2201	synthetic cannabinoid; 1-(5-fluoropentyl)-3-(1-naphthoyl)indole
CI	chemical ionisation
CNS	central nervous system
mCPP	<i>meta</i> -chlorophenylpiperazine or 1-(3-chlorophenyl)piperazine
3,4-DMMC	3,4-dimethylmethcathinone
DDD	Defined daily dose. The assumed average maintenance dose per day for a drug used for its main indication in adults
2-DPMP	desoxypipradrol or 2-benzhydrylpiperidine or 2-diphenylmethylpiperidine
DRD	drug-related death
DUI	driving under the influence (of alcohol or drugs or both)
DUID	driving under the influence of drugs
ECD	electron capture detection
EI	electron ionisation
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EWS	early warning system
FIMEA	Finnish Medicines Agency
GABA	<i>gamma</i> -aminobutyric acid
GC	gas chromatography
GHB	<i>gamma</i> -hydroxybutyric acid
HP-GC-FID	headspace gas chromatography with flame ionisation detection
HRMS	high-resolution mass spectrometry
JWH-018	synthetic cannabinoid; 1-pentyl-3-(1-naphthoyl)indole
LC	liquid chromatography
LLE	liquid-liquid extraction
MDEA	3,4-methylenedioxy- <i>N</i> -ethylamphetamine
MDMA	3,4-methylenedioxy- <i>N</i> -methylamphetamine or ecstasy
MDPV	3,4-methylenedioxypyrovalerone or 1-(3,4-methylenedioxyphenyl)-2-pyrrolidino-1-pentanone
MePPP	4'-methyl- α -pyrrolidinopropiophenone
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NPD	nitrogen-phosphorus detection
NPS	new psychoactive substance
PM	post-mortem
PMA	<i>para</i> -methoxyamphetamine
PMMA	<i>para</i> -methoxy- <i>N</i> -methylamphetamine
SPE	solid phase extraction
<i>t.i.d.</i>	three times daily (lat. <i>ter in die</i>)
$t_{1/2}$	elimination half-life
TOFMS	time-of-flight mass spectrometry
UNODC	United Nations Office on Drugs and Crime
V_d	volume of distribution

ABSTRACT

Driving under the influence of drugs (DUID) can adversely affect driving skills in numerous ways and put lives at risk. Legal approaches to DUID vary considerably from country to country, even within Europe, and, in the last decades the emergence of new psychoactive substances (NPS) has further complicated the scene. DUID is an unlawful act if the substance taken is banned or impairs driving. The latter is hard to define and prove, putting pressure on governments to ban NPS as quickly as possible in order to protect the public by facilitating enforcement of DUID laws. However, banning requires knowledge on several aspects of NPS such as prevalence, pharmacology, abuse potential and toxicity. Up-to-date, evidence-based information on NPS is needed by legislators, toxicologists, clinicians, and other health care professionals. Such information would enable potential drug users and the public to be more aware of the risks associated with illicit use of NPS. This study aimed to add to the knowledge of the NPS most relevant in Finland.

In this thesis, the prevalence, blood concentrations in drivers and in post-mortem cases, and demographic details of 3,4-methylenedioxypyrovalerone (MDPV) and desoxypipradrol (2-DPMP), were investigated. Changes in prevalence and other characteristics of MDPV were monitored over a time span covering a period before its banning as well as a few years after banning. Phenazepam, a Russian therapeutic benzodiazepine now illegal in Finland, was studied by examining both DUID and post-mortem cases. The use by apprehended drivers of pregabalin, a prescription anticonvulsant with therapeutic indications for neuropathic pain, partial seizures and generalised anxiety disorder, was also studied.

The results of this study showed that DUID cases provide a valuable source of information on NPS prevalence and user profiles. However, little specific information could be gained about the impact on driving performance and health risks of NPS mainly due to the fact that NPS were usually used together with a spectrum of other psychoactive substances. It could, however, be concluded that all of the studied NPS were frequently detected in the samples collected from apprehended drivers and, in the case of MDPV, the prevalence changed with time. The number of MDPV-positive cases among apprehended drivers decreased by 51.1% after the drug was banned. The concentrations of NPS found in DUID cases were within the range anticipated to produce significant adverse effects on driving performance, or, in some cases, in the range found in post-mortem cases where the drug may have contributed to the fatality. The presence of the medicinal drug, pregabalin, was found to be connected to abuse rather than appropriate medical use since it was in most cases found in concentrations higher than those recommended for therapeutic use and together with illegal drugs such as amphetamine or cannabis. In post-mortem cases positive for MDPV, the prevalence of suicide was much greater than in fatalities related to other drugs.

Three independent registries, namely the DUID toxicology data, the post-mortem toxicology database, and court documents, were examined to gain novel information on the characteristics of NPS use and those abusing them. The large number of cases studied produced information on concentration ranges associated with abuse of the studied substances.

1 INTRODUCTION

The last ten or twenty years have brought tremendous change to the illicit drug market. The rapid emergence of new psychoactive drugs (NPS) is a growing problem all over the world and constantly creates challenges to toxicologists and health care professionals dealing with drug users. One of the reasons for the popularity of these drugs is the widespread availability and easy access to the substances through the Internet. Drug users seem to be willing to try substances that have gained attention in the media and in Internet forums despite the lack of scientific data on the potential harm they may cause.

According to the United Nations Office on Drugs and Crime (UNODC), NPS are defined as “substances of abuse, either in pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat” [1]. Probably the best known group of NPS are synthetic chemicals (“designer drugs”) that have been designed to imitate the structure and/or effects of more established drugs. They may initially have been discovered a long time ago while developing new molecules for medicinal use. The motivation for manufacturing such substances in the present context is to circumvent the legal consequences associated with the traditional illegal drugs. In late 1970s, more and more clandestine laboratories started synthesizing “designed” substances that resembled controlled or banned substances but were not yet regulated. This manufacturing included, and still includes, nearly all types of drugs from opioids to phencyclidine analogues [2]. Manufacturing these substances is relatively easy, their distribution and ordering via the Internet is simple and the legal consequences if caught are mild or non-existent. The risk of being caught is very low due to the fact that many NPS cannot be screened for right after their introduction to the drug market since analytical methods and reference material are usually not available. Consequently, the constant demand of new drugs and the ease of manufacturing them have led to a vicious circle in which authorities struggle to ban new substances while manufacturers create new substances at an accelerating pace (Fig. 1).

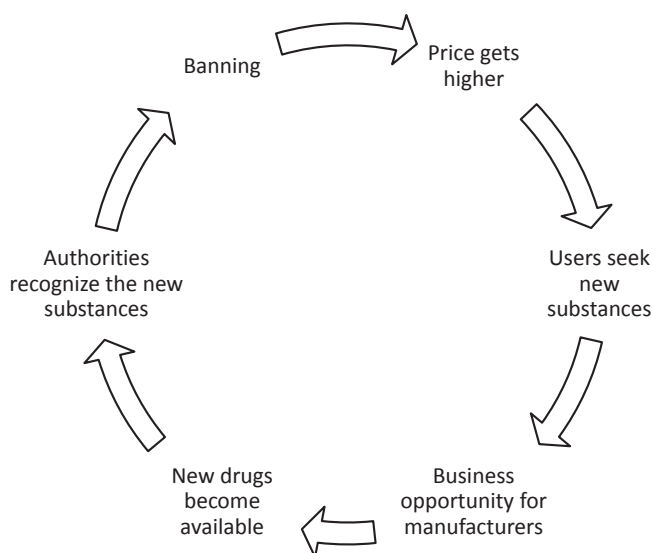


Figure 1. The vicious circle of NPS.

Many of the NPS are stimulants, such as cathinone derivatives resembling the original substance obtained from chat (*Catha edulis*, khat), and several possess hallucinogenic properties. Synthetic cannabinoids have also gained much exposure in the communities of illicit drug users in recent years, albeit less in Finland. In addition to synthetic chemicals, the term “NPS” also includes abused plant derived substances such as mitragynine and *Salvia divinorum*.

Driving under the influence of drugs (DUID) has a major detrimental impact on traffic safety in Finland and all over the world. In Finland, a drug analysis is requested in about 4000 traffic cases every year. At least one psychoactive substance is detected in over 90% of the cases. Since all Finnish DUID samples are analysed in one central laboratory and reported to the National Bureau of Investigation, the analysis results form an excellent source to explore the prevalence of different drugs.

The zero-tolerance law for illegal drugs in DUID was introduced in 2003 in Finland. Among other things this means that the driver does not need to show signs of impairment but a suspicion (in most cases that of a police officer) is sufficient for bringing the suspect for a blood test. The tests for impairment or road-side sobriety tests, that in many countries are mandatory before proceeding to further examinations, have been shown to be inefficient in detecting driving impairment, especially in cases of stimulant use [3,4,5]. Tolerance can be formed towards some elements of the drug effects, which can further complicate the interpretation of sobriety tests. Some countries, e.g. Norway, have set penalties that are graded according to the levels of different drugs found in blood [6]. In Finland, the zero-tolerance law means that a person is not allowed to operate a motor driven vehicle after consuming substances listed in the Narcotics Act in such a manner that, during or after driving, the substance at issue or its metabolite is detectable in the blood of the driver [7]. If the drug in question is approved for medical use in Finland and the driver has a valid prescription for the drug, the blood concentration must be within the therapeutic range and the person should not show signs of impairment. Before having been banned, most NPS fall outside of this law. Drivers with unbanned substances usually escape prosecution for DUID because of the difficulties in making a legally valid determination of driving impairment. Even though the authorities are diligently working on controlling new drugs, the manufacturers seem always to be a couple of steps ahead. The sooner the necessary scientific information is available to the authorities on the new substances, the sooner the process of banning can be started.

In addition to DUID cases, knowledge of NPS is especially valuable in toxic emergencies, in the treatment of drug addicts and in post-mortem investigations. If no information is available to the clinician or forensic pathologist on the substances present in their cases, it is difficult to assess the relevance of the substance for the case.

Data obtained from hospital discharge registries are incomplete because non-fatal poisoning cases are seldom confirmed by laboratory analysis in Finland. In contrast, toxicological results from Finnish post-mortem investigations form a comprehensive database covering all the analytical results from the nearly 13% of deceased every year that undergo a thorough toxicological analysis in connection with the medico-legal examination. Comparing this data with the DUID material gives valuable insight into the drug situation in Finland.

In the present study, the prevalence and significance of certain selected NPS were examined in DUID cases in Finland. For comparison, the post-mortem cases positive for these substances were assessed. These substances were among those added to the list of drugs screened for in routine DUID procedures because Finnish Police and Customs were reporting their detection in material confiscated from importers or dealers of illegal drugs and it seemed likely they were

entering the local recreational drug marketplace. Of the NPS that were included in the screening, the substances selected for inclusion in this thesis were the ones that had been frequently detected in the samples from the apprehended drivers. The studied substances include two stimulant NPS (MDPV and 2-DPMP), and two sedatives, including one older benzodiazepine molecule that is not in medical use in the western world (phenazepam) and one medicinal drug that has recently been noticed to possess considerable abuse potential (pregabalin).

By developing the techniques for the monitoring of drug use, traffic safety can be improved by means of informing drivers of the risks associated with recreational use of drugs and also by enabling surveillance and legislation. People working with drug addicts need information about geographical distribution of NPS usage. National and international drug prevention and early intervention programs greatly benefit from new information about NPS and medicinal drug abuse.

Given the multitude of aspects and problems associated to of the use of new, untested and uncontrolled psychoactive substances, scientific studies on NPS are crucial for many reasons:

- Health care professionals need evidence-based and up-to-date information when treating patients abusing NPS.
- Forensic toxicologist and pathologists need information on prevalence, blood concentrations and drug use patterns when developing new methods and interpreting analytical results.
- Government authorities need all the available information for regulatory purposes as well as for planning intervention strategies.
- The public needs to be informed about the risk associated with the use of NPS.

Understanding differences in drug use patterns can help in recognising drug users and the problems connected to different forms of addiction.

2 REVIEW OF THE LITERATURE

2.1 Drug use, abuse and addiction

UNODC has estimated that about 243 million people (5.3% of all 15-64 year olds), took illicit drugs at least once in 2012 [1]. Much less information is available about NPS prevalence and user profiles [8]. For example, it is not known whether the users of NPS also use the more established, banned or controlled, older drugs, or, whether there are users who experiment with new substances in order to avoid the legal consequences associated with illegal drugs or to gain new experiences. NPS have in any case clearly established their presence in the drug scene. In 2014, 101 completely new psychoactive substances were reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), raising the number of monitored substances to more than 350 [9].

Even though Finland is a small country at the far edge of the western world, a surprisingly large number of newly detected substances are reported to the EMCDDA by the Finnish authorities every year. A large amount of information on new emerging drugs can be obtained from the reports of drugs confiscated by Customs. In 2013, the Finnish Customs detected about 100 different NPS of which 20 were detected for the first time [10]. The most important group of NPS in terms of prevalence and problems for users in Finland have been the new designer stimulants. The history of stimulants being very popular in Finland and other Nordic countries has apparently created a culture where NPS are welcomed and appreciated by users of illegal drugs [11].

According to statistics submitted to EMCDDA concerning the year 2012, 2.3% of Finns aged 15–64 years had used amphetamines at some time in their lives; a prevalence that was lower than the mean for the European Union (3.4%) and also lower than in Sweden (5.0%) and the UK (10.6%). However, the 2012 data also showed that prevalence of use in the previous 12 months among Finnish youth and young adults aged between 15–34 (1.6%) was higher than in any of the aforementioned countries (EU 0.9%, Sweden 1.5%, UK 1.1%) [12]. Among those in Finland seeking treatment for their drug use, for 80.4% the main route of amphetamine administration was by injection, which also is higher than the mean for the EU (48%) or for Sweden (76.5%) and the UK (26.1%) [12].

Most researchers acknowledge the following three stages in the use of drugs: (1) recreational drug use – meaning sporadic use one or several drugs, (2) escalated drug use, and (3) addiction – meaning the loss of control in respect of drug use [13]. Recreational drug use means the use of a drug (legal or illicit) with the intention of achieving positive feelings. However, most recreational drug users do not become drug addicts [14]. For a sub-set of recreational users, what starts as voluntary drug use or isolated experiments with drug use, may eventually proceed to compulsive drug use (addiction).

Drug addiction or substance dependence is a serious social problem creating costs and suffering for those using drugs, the people around them and society. As defined by the diagnostic criteria in the International Classification of Diseases (ICD 10th edition), dependence syndrome includes at least three of the following:

1. A strong desire or sense of compulsion to take the substance,
2. Difficulties in controlling substance-taking behaviour in terms of its onset, termination, or levels of use,
3. A physiological withdrawal state when substance use has ceased or been reduced,
4. Evidence of tolerance,
5. Progressive neglect of alternative pleasures or interests because of psychoactive substance use, increased amount of time necessary to obtain or take the substance or to recover from its effects,
6. Persisting with substance use despite clear evidence of overtly harmful consequences [15].

In the similar classification by the American Psychiatric Association used in the US, the former disorders “substance abuse” and “substance dependence” have been combined into a new concept “Substance use disorder” (SUD) [16].

It has been suggested that the so-called “normalisation” of the use of certain psychoactive substances that has occurred in recent years has reduced the barriers to experimenting with drug use [17]. However, the major factors driving drug use can be roughly categorised into: (1) the urge to feel good and (2) the need to feel better. The first, “positive re-enforcement”, results in a pleasurable “high” which users wish to experience repeatedly. The second, “negative re-enforcement”, can be seen as “self-medication” of negative feelings or other conditions [18,19,20]. Sometimes self-medication is needed to control the side-effects of other recreational drugs.

According to one of the largest surveys of recreational drug use ever conducted, in which over 40 000 Americans were asked questions about alcohol and drug use and related disorders, 2% of the study population met the criteria for drug use disorder [21]. In an earlier Finnish study, “problem drug users” were defined as individuals that have taken amphetamine or opioids in a manner that caused adverse social or health effects that in turn prompted intervention by the authorities resulting in the individual’s name having been entered in a national official register (e.g. hospital discharge register, the national police information system, the register of drivers caught driving under the influence of drugs, hepatitis C cases in the national infectious diseases register) [22]. The number of such problem drug users in Finland in 2012 was estimated to be between 18 000 and 30 000 individuals corresponding 0.55–0.90% of the population aged between 15 and 64 [22].

Substance dependence also impacts driving in several ways, one of which is that an individual who fulfils certain drug-use criteria is not permitted to possess a driving licence. According to the EC Directive on driving licences, an applicant who is dependent on psychotropic substances or regularly uses them shall not be issued a driving licence [23]. It remains for the member states to decide how the fitness to drive is tested or which criteria are used in assessing possible substance dependence.

In Finland, the prerequisites for being fit to drive are regulated in various laws. The request for a physician to evaluate the fitness to drive of a suspected drug-dependent individual is made by the Police who also make the final decision of whether or not the person is granted a driving licence [24]. However, a significant proportion of DUI offenders do not possess a valid driving licence and are thus outside of this evaluation process.

2.2 New psychoactive substances

The number of NPS reported yearly to the EMCDDA has grown every year since 2008 [9]. In many countries, NPS are sold in so called “head shops” and the drugs are sometimes labelled “not for human consumption” [25,26]. They may be marketed as “bath salts”, “plant food” or “herbal incense” in order to circumvent the law. In Finland, however, the main source of NPS has always been the Internet and the drugs are mainly purchased as “research chemicals” or transported for personal use from abroad [12,27]. At present, there is very little scientific data available on the prevalence of NPS in Finland.

In the last few years, NPS markets in the “darknet” or “deep net” has become an issue. “Darknet” is a series of anonymised websites that users with special knowledge can access in order to buy illegal products such as drugs, guns, pornography etc. Transactions are encrypted and payments made with Bitcoins, thus assuring anonymity of both parties [28,29].

All new stimulant drugs, as is the case with the more established illegal stimulants amphetamine, cocaine and ecstasy (MDMA), act by increasing the levels of extracellular neurotransmitters dopamine, serotonin and noradrenaline in the central nervous system (CNS) [30]. The increased levels of these neurotransmitters mediate the behavioural effects of stimulants such as hyperactivity, euphoria, and analgesia. In low doses, many of the synthetic cathinones have entactogenic effects [31]. Especially in the US, NPS have become popular in mixtures called “psychoactive bath salts” which may contain just one substance or a mixture of a number of psychoactive drugs. The ingredients of “bath salts” and other “legal highs” that are marketed under a certain brand name may change over time [32].

In addition to the effects that the users seek, the structures of many of the NPS predict severe adverse effects especially when taken repeatedly and in high doses. Most reports on the adverse effects of NPS involve abuse of synthetic cathinones. The most common clinical symptom in severe intoxication cases resulting in hospitalisation has been acute psychosis but also the following have been frequently reported: paranoia, visual and auditory hallucinations, self-injurious behaviour, homicidal thoughts, and amnesia [33,34]. Some less prevalent effects have included hyponatremia, cerebral oedema and hypoglycaemia [35,36]. By enhancing sympathetic nervous system activity, synthetic cathinones can cause cardiovascular effects such as tachycardia, hypertension and thrombosis [37,38]. The increased serotonergic activity can cause serotonin syndrome with the following symptoms: severe agitated delirium, life-threatening hyperthermia, rhabdomyolysis, and renal failure [39,40,41].

Since new substances enter the recreational drug marketplace at such a fast pace, research on them usually comes later. So far, research on NPS has mostly been limited to case reports, measurement of drug levels in community wastewater, custom’s reports on confiscated drugs, poison centre data and analysis of pooled urine from portable urinals [42,43,44].

One of the many problems associated with the use of NPS is that the users often do not know which drug(s) they are taking [45,46,47]. Either the drug has been sold to them as something else or they may have purchased a drug with a “brand name” such as “Ivory Wave” or “Super Coke” which are mixtures whose content varies with time due to changes in legislation and other factors [32]. As potency can vary significantly between different psychoactive substances, selection of the appropriate dosage of substances of unknown composition is guesswork and may bring about stronger or unwanted effects, or toxicity, making this a very dangerous phenomenon. Thus, from the users’ perspective, a major risk in taking NPS is dose selection [48]. Since users cannot be sure of the identity or dose of the substances they use, the value of survey-type research on NPS

that does not involve analysis of biological specimen to identify the drugs and doses involved is limited.

The motivation for taking NPS, as opposed to older psychotropic substances, probably varies between individuals but may include e.g. the need to circumvent the legal system by ordering or using uncontrolled substances, or curiosity. The desired effects may include e.g. feeling of increased energy and vigilance, increased libido, empathy, euphoria, alertness and well-being and psychedelic effects [25,49,50,51].

2.2.1 Non-medical prescription drug use

NPS have also been defined more broadly as any substance that has come to the illegal drug marketplace within the last 10 to 20 years or where there is evidence of significant increase in abuse of a substance in recent years [52]. Hence the term also includes many abused prescription drugs that were not designed for recreational use but rather were developed and approved by regulatory authorities to treat specific health issues. With time, those medicinal products with appropriate pharmacological properties may gain a reputation among users of illegal drugs and eventually become abused. This is unfortunate since restricting the availability of medicinal drugs due to problems with abuse and dependency always complicates the lives of those patients who benefit from the therapeutic effects of the drug.

Several different terms are used to describe medical uses of prescription drugs which were not specifically endorsed by regulators when the drug was approved to treat disease. “Off-label use” means the therapeutic use of the drug, as prescribed by a physician, for an indication, age group, dosage etc. that is not listed in the approved documentation (label) for the drug. Off-label use is legal and medically acceptable if pursued on the initiative and under the supervision of a physician in the best interest of the patient. “Misuse” of prescribed drugs is use in an effort to treat the condition for which the drug was described but in ways that were not intended by the prescribing physician. Ways of misusing medicinal products are only limited by the imagination of the user; it can include use of higher doses, more frequent dosing, using the drug for longer periods than intended, changing the routes of administration, or inappropriate concomitant use of other drugs [53]. Misuse can have many causes, including ignorance or error on the part of the patient, or an effort to achieve a greater therapeutic effect. “Drug abuse” or abuse of medicinal drugs refers to non-prescribed use of one or several drugs in order to achieve certain pharmacological effects; frequently effects on the CNS such as improved mental state or psychomotor performance. The desired effects most often include tranquillity, euphoria or increased alertness. Less common motives for “self-medication” may be achieving weight loss, treating insomnia or some undiagnosed medical condition. An additional reason for abusing prescription drugs may be to counteract the negative side-effects or come-down effects of other drugs of abuse [54].

Epidemiology of non-medical prescription drug use is often studied by conducting population surveys. In Finland, the last national survey on drug use concerning the year 2010 revealed that 6% of all Finns aged between 15 and 69 had ever used sedative, hypnotic or analgesic drugs for non-medical purposes [55]. Among the individuals seeking treatment for problems related to drug abuse, the percentage of those with sedative medicinal drugs as a primary drug of abuse has increased in recent years and was 60% in 2013 [10].

2.2.2 Chemistry of NPS

New drugs can be grouped in several different ways such as according to the older abused drug they resemble, for example designer amphetamines, designer opioids or synthetic cannabinoids. The structure-based categorization of NPS used by EMCDDA suggests the following groups:

- synthetic cathinones: synthetic derivatives of cathinone which is one of the stimulant ingredients of the shrub khat (*Catha edulis*) [56],
- synthetic cannabinoids: chemicals that are designed to mimic the effects of tetrahydrocannabinol, the main active ingredient of cannabis [57],
- piperazines: a group of chemicals with a piperazine functional group producing mostly stimulant/euphoric effects [58],
- tryptamines: hallucinogenic compounds structurally related to LSD [59],
- phenethylamines: a large group of substances that may produce stimulant, psychedelic and entactogen effects [60].

The vast majority of the NPS fall into the first three categories [61]. Some of the NPS do not fall in any of these classes and are thus categorised as “other” including e.g. plant material and medicinal products.

2.3 Analytical aspects of NPS

Conventional laboratory and roadside drug testing has been designed to detect the traditional abused drugs, namely, amphetamines, cannabis, cocaine, and heroin. Additionally, buprenorphine and benzodiazepines may be included. Many of the test batteries in clinical and hospital laboratories consist of immunological analyses which are an efficient way of rapidly screening a large number of samples, without complicated sample pre-treatment procedures and at moderate cost per sample. The immunological tests are especially useful in situations where the initial assumption is that the majority of the samples will be negative. An example of such a situation is workplace drug testing.

Despite the advantages of immunological methods for screening large numbers of samples, they have a number of limitations. In cases with potential legal consequences, all positive results from immunological testing must be confirmed by another method. Even though the modern immunoassays have been shown to cross-react with some of the NPS [62], current methods do not detect the range of analytes relevant in today's toxicology. Immunoassays are in most cases not able to detect the ever expanding variety of NPS that include substances from multiple pharmacological categories [63]. Additionally, many abused prescription drugs are not detected by current immunological methods. Furthermore, the detection limits of immunological tests are often much higher than those in chromatography-based screening methods and thus some individuals with low drug concentration may escape detection if screened immunologically.

In many forensic indications, the analytical approach most suited to the screening of a large and varying spectrum of abused drugs is liquid chromatography (LC) combined with high-resolution mass spectrometry (HRMS) [64,65,66,67]. Time-of-flight MS (TOF-MS) methods have been found to be particularly useful since they incorporate the accurate mass of precursor and qualifier ions, isotopic pattern, and retention time to provide a positive identification. LC-TOFMS also enables the preliminary identification of substances for which a reference standard is not available [68].

2.4 Substances selected for investigation in this thesis

The NPS studied in this thesis were MDPV, phenazepam, 2-DPMP and pregabalin. Details of typical doses, blood concentrations and pharmacology of these substances are given in Table 1.

Table 1. Pharmacological details of the studied substances.

	Typical recreational dose (mg)	Ther. range in plasma (mg/L)	DDD (g) ^a	T _{1/2} (h) ^b	V _d (L/kg) ^c	References
MDPV	5–20	-	-	1.88	-	20,69
Phenazepam	0.5–2.0	0.030–0.070	-	60	4.7–6.0	70,71,72,73
2-DPMP	1–10	-	-	16–20	-	72
Pregabalin	150–800	2.0–8.0	0.3	5–11	0.5–0.6	72,74

^adefined daily dose (the assumed average maintenance dose per day for a drug used for its main indication in adults), ^belimination half-life, ^cvolume of distribution

All previous information published in peer-reviewed journals about blood concentrations after recreational use of the four substances is collected into Table 2. For some drug screening indications, such as testing individuals in drug rehabilitation programs, the primary specimen for the analysis is urine. These cases were not included in Table 2. The material used for analysis in the post-mortem studies varied in the reviewed articles and in some cases samples of multiple tissues had been analysed. In some studies, the origin of the post-mortem blood sample was not mentioned. In cases where results were available from several tissues, the results from femoral blood are listed in Table 2.

Table 2. Published blood concentrations of abused drugs in samples from living individuals and from post-mortem (PM) investigations. DUID cases are marked with an asterisk (*).

Substance	Concentration (mg/L)	Specimen	N	Reference
MDPV living				
	0.024 – 0.241 (mean 0.058)	Serum	13	75 (Spiller et al. 2011)
*	0.006 – 0.368 (mean 0.100)	Blood	8	76 (Marinetti et al. 2013)
	0.186	Serum	1	77 (Thornton et al. 2012)
*	0.0213 and 0.146	Serum	2	78 (Maas et al. 2015)
	0.072 – 0.089	Blood	3	79 (Froberg et al. 2014)
	0.124 and 0.306	Blood	2	80 (Adamovicz et al. 2013)
*	<0.01 – 0.30 (median 0.05)	Blood	33	81 (Griffiths et al. 2015)
MDPV PM				
	0.039 and 0.760	PM blood	2	82 (Wright et al. 2013)
	0.17	PM blood	1	75 (Spiller et al. 2011)
	0.082	PM blood	1	26 (Murray et al. 2013)
	0.44	PM blood	1	83 (Wyman et al. 2013)
	0.010 – 0.640 (mean 0.109)	PM blood	16	76 (Marinetti et al. 2013)
	0.06 – 1.55	PM blood	8	84 (Elliott et al. 2014)
	1.0	PM blood	1	85 (Kesha et al. 2013)
	0.0222	PM blood	1	86 (Tóth et al. 2013)
	0.017 and 0.038	PM blood	2	80 (Adamovicz et al. 2013)
	1.2	PM Blood	1	87 (Namera et al. 2013)
Phenazepam living				
*	0.076	Blood	1	88 (Kerrigan et al. 2013)
*	0.04 – 3.2 (median 0.17)	Blood	11	89 (Stephenson et al. 2013)
	1.2 µg/g	Blood	1	90 (Mrozkowska et al. 2009)
*	0.120 – 0.870	Blood	3	91 (Burch et al. 2013)
	0.49	Serum	1	92 (Dargan et al. 2013)
Phenazepam PM				
	0.386	PM blood	1	93 (Bailey et al. 2010)
	2.52	PM blood	1	94 (Corkery et al. 2015)
	0.009 – 0.370 (median 0.076)	PM blood	23	95 (Crichton et al. 2015)
2-DPMP living				
*	0.356	Serum	1	78 (Maas et al. 2015)
2-DPMP PM				
	0.025 – 1.16	PM Blood	3	96 (Corkery et al. 2012)
Pregabalin living				
	29	Blood	1	97 (Grosshans et al. 2010)
Pregabalin PM				
	0.28 – 110	PM Blood	316	98 (Häkkinen et al. 2014)
	0.04 – 23.8 (median 5.18)	PM Blood	43	99 (Lottner-Nau et al. 2013)
	0.4 – 17.0 (median 5.6)	PM Blood	15	100 (Priez-Barallon et al. 2014)

2.4.1 MDPV

In Finland, one of the most prevalent NPS has been MDPV. It belongs to the chemical class of α -pyrrolidinovalerophenones and is a methylenedioxy-substituted analogue of the older medicinal stimulant drug pyrovalerone [101]. MDPV was first developed by the researchers of the pharmaceutical company Boehringer Ingelheim in 1969 [102]. MDPV and other cathinone derivatives are structurally closely related to amphetamines – especially MDMA – differing mainly by a β -keto amine group. Cathinone derivatives such as MDPV, mephedrone and methyldone are often called β -ketoamphetamines (Fig. 2) [103]. Based on their structure, synthetic cathinones can be predicted to have similarities in their pharmacological profile to MDMA analogues [33].

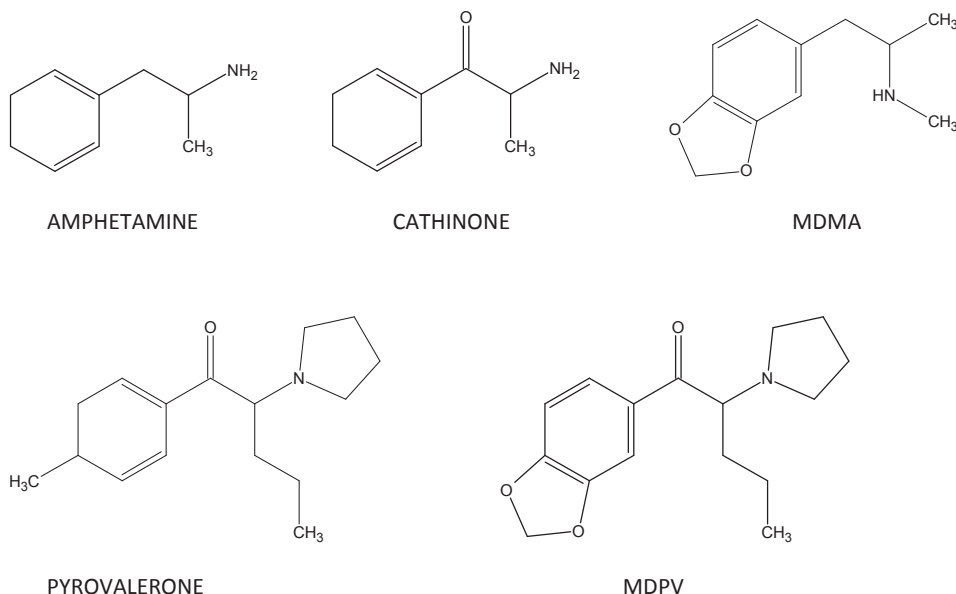


Figure 2. Chemical structures of amphetamine and some cathinones.

MDPV became available for the users of illegal drugs in Finland around 2007, after having been identified in recreational drug users elsewhere in Europe some years before [101]. In Finland, MDPV was scheduled as a narcotic in 2010 – a process that prompted the amendment of the Narcotics Act that now allows for national scheduling without previous listing by other authorities, e.g. the United Nations. MDPV is currently banned in all member states of the EMCDDA as well as classified as a Schedule I substance by the US Drug Enforcement Administration. Currently, MDPV is under consideration by the Commission on Narcotic Drugs (UN) for being placed in Schedule I of the Single Convention on Narcotic Drugs [104].

In animal and in-vitro studies, MDPV has been shown to be a potent blocker of dopamine re-uptake [105,106,107] suggesting a high risk for abuse for the drug by contributing to the drug-high and euphoria that are the effects that users desire [15]. It is also a potent blocker of norepinephrine uptake, which predicts severe cardiovascular effects [105]. In severe intoxication cases, the following symptoms have been reported: severe agitation, psychosis, violent behaviour, hyperthermia, tachycardia, severe serotonin syndrome, organ failure such as rhabdomyolysis,

hepatic and renal failure, delusions, hallucinations and paranoia [40,75,108,109]. Deaths have also been reported [26,40,Table 2].

The metabolism of MDPV has been shown to involve multiple different CYP isoenzymes and thus the existence of complications from genetic polymorphism or significant drug-drug interactions is unlikely [110].

In recent studies on community wastewaters from cities around Finland it was shown that MDPV was quite prevalent in eastern parts of Finland close to the Russian border [111,112]. In one of the studies that measured drugs from wastewaters in 9 different water treatment plants in 2012, MDPV was exclusively found in the South-eastern city of Savonlinna [111]. In the other similar 2012 study, water from 10 treatment plants was examined and MDPV was detected in another south-eastern city, Lappeenranta, and also in Helsinki albeit in lower amounts [112].

2.4.2 Phenazepam

Phenazepam (Fenazepam, “Bonsai”) is a long-acting benzodiazepine that was originally developed in the former Soviet Union in the 1970s [113]. It has never been in medicinal use in Finland nor in other western countries, but is still widely prescribed in Russia and some former Soviet countries. Phenazepam was banned in Finland in July 2014.

Phenazepam belongs to the group of 1,4-benzodiazepines. It is structurally similar to other benzodiazepine derivatives, for example, diazepam, lorazepam and nitrazepam (Fig. 3).

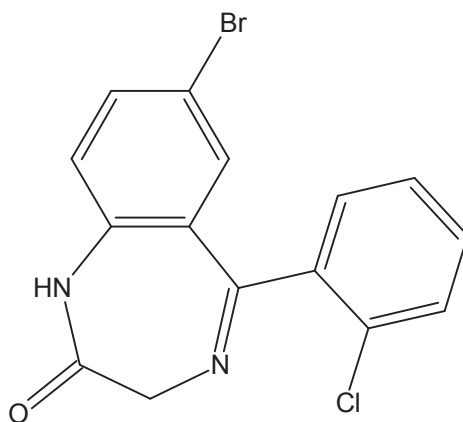


Figure 3. The chemical structure of phenazepam (7-Bromo-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one).

Since around 2009, a growing number of reports of phenazepam abuse have been published in several countries, e.g. USA, UK, New Zealand, Sweden and Finland [114,115,Table 2]. Fatalities have also been reported [11,116,117, Table 2]. Phenazepam has been found as a component in mixtures of two or more psychoactive substances, such as with synthetic cannabinoids [115].

According to several anecdotal user reports in Internet forums, phenazepam is not widely used for self-medicating anxiety (unlike e.g. alprazolam) but is rather used for getting “high”. The users have estimated the potency of phenazepam to be approximately five times that of diazepam.

Similar to other benzodiazepines, phenazepam can slow reaction time and cause drowsiness and confusion in drivers [89]. Its long half-life means that adverse effects persist and may

accumulate when re-dosed [88]. Phenazepam has also been suggested to induce amnesia, at least at higher doses [Internet forums, 89].

2.4.3 Desoxypipradrol

Desoxypipradrol (2-DPMP, “Daisy”) is an example of the new designer stimulants that are actually not so new at all. The substance was originally in development for the treatment of narcolepsy and Attention Deficit Hyperactivity Disorder (ADHD) in Switzerland as early as in the 1950s [118] but was later withdrawn from development. The second appearance of the drug started in 2007 when the users began to discuss a new, extremely long-acting substance in their Internet forums. In Finland, the first reports of the drug date back to 2008 and it was finally banned in 2012.

As the name indicates, desoxypipradrol is a desoxy analogue of pipradrol (Fig. 4) which is an old CNS stimulant that is not considered to be particularly potent in terms of its stimulant properties. Pipradrol resembles amphetamine in its mechanism of action but differs from it by not causing insomnia, inappetence or post-use depression [119]. It is still used in some countries (e.g. South-Africa) under the trade name Alertonic or Meratran to treat conditions like narcolepsy, ADHD and symptoms of dementia.

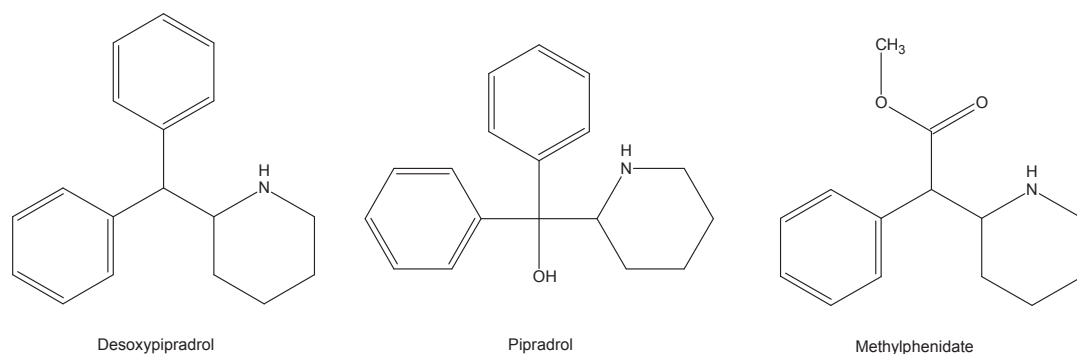


Figure 4. The chemical structures of desoxypipradrol [(*RS*)-2-benzhydrylpiperidine], pipradrol (α,α -diphenyl-2-piperidinemethanol) and methylphenidate [methyl 2-phenyl-2-(piperidin-2-yl)acetate].

Desoxypipradrol is structurally similar to methylphenidate (Fig. 4) but it is much more potent and long-acting [120]. Contrary to methylphenidate, desoxypipradrol cannot be eliminated by ester hydrolysis but undergoes complex metabolism [121]. Desoxypipradrol acts by increasing the release of dopamine and slowing its re-uptake and its effects are suggested to be greater than those of cocaine [122].

The popularity of desoxypipradrol as a recreational drug seems to be very regional. There are only few case reports of desoxypipradrol use from other countries [123,124, Table 2] whereas in Finland it gained massive publicity in 2010 when several young people developed severe side-effects from using the initially unknown substance called “Daisy”.

According to some discussions on Internet user forums, the long half-life of desoxypipradrol makes it popular with individuals trying to remain alert through the night, such as students or over-night drivers.

2.4.4 Pregabalin

Pregabalin is a relatively new medicinal drug that is widely used around the world for the treatment of epilepsy, neuropathic pain and fibromyalgia. It has been available for medicinal use since 2004. For quite some time it was thought to lack any potential for abuse, which led to suggestions that it would be useful for the treatment of anxiety and other indications in drug addicts and in alcohol detox [125,126]. Pregabalin has been approved for the treatment of generalised anxiety syndrome in Europe since 2006 but is still not approved for that indication in the US. In the last few years, reports of misuse and abuse have appeared [98,127,128,129, 130,131,132, Table 2] and today pregabalin is a prime example of prescription drug abuse.

Pregabalin (Fig. 5) is a structural analogue of *gamma*-aminobutyric acid (GABA) and, although inactive at all GABA receptors, it possesses GABA-mimetic properties [129,133]. It reduces the release of neurotransmitters, such as noradrenaline, glutamate and acetylcholine from synapses thus producing its therapeutic effects: prevention of seizures in the treatment of epilepsy, analgesia and anxiolytic activity. The latter are similar to those induced by benzodiazepines or alcohol and can potentially be of interest for the users of recreational drugs [133,134]. Additionally, pregabalin has been shown to induce euphoria in some individuals [135,134] and to potentiate the effects of opioids [98].

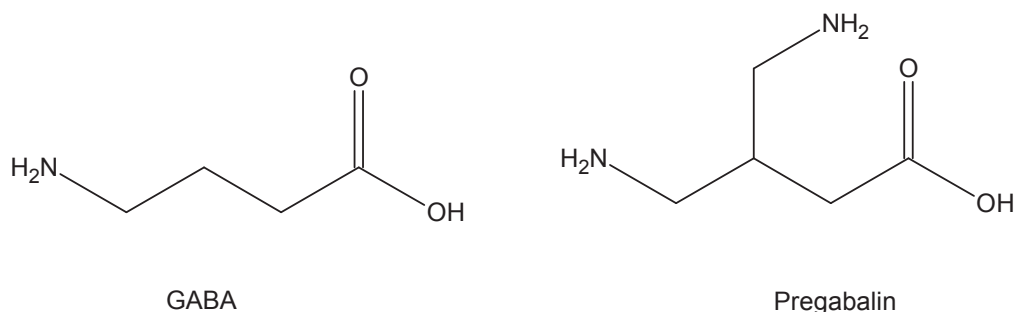


Figure 5. The chemical structures of GABA (4-aminobutyric acid) and pregabalin [(S)-3-(aminomethyl)-5-methylhexanoic acid].

The effects of pregabalin on driving have been studied in driving simulator studies with healthy volunteers but no major effects were observed [136,137]. In studies of Finnish drugs users, the frequency of pregabalin detection in post-mortem investigations has been steadily increasing [11, Table 2].

2.5 Driving under the influence of drugs

It has been estimated that of the general population of car drivers in Europe at any given time, 1.90% drive under the influence of illicit drugs [138]. There are, however, major differences in the numbers between individual countries, even among neighbouring countries, due to cultural differences and differences in law enforcement and prescription policy [139,11].

In Finland, the majority of DUI cases arise from a traffic violation, e.g. accidents or failure to follow the traffic rules [140]. Every year in Finland, over 20 000 drivers are apprehended for

driving under the influence of drugs or alcohol. In the majority of the cases, the substance used is ethyl alcohol. In about 4000 DUI cases yearly the suspicion is of drugs.

2.5.1 Screening and laboratory analytics in DUI cases

As per the instructions of the Ministry of the Interior the primary method for confirming suspected driving under the influence of alcohol is the evidential breath analyser. In cases of suspected drug use, the driver is usually first subjected to a road-side oral fluid drug screening test (e.g. DrugWipe®) and later a blood test is taken in the nearest health centre by a physician or phlebotomist. Even though urine is the preferred analysis material in many clinical and forensic settings, in DUID cases, the primary material for analysis in most countries is blood or serum. For screening purposes, oral fluid is increasingly being used due to the non-invasive nature of sample collection [141]. Blood and oral fluid samples can be collected shortly after a driving incident occurred and drug levels measured in such samples have a direct bearing on impairment at the time of the incident. In contrast, drug levels measured in urine reflect blood levels over an extended window of time that includes a period prior to the driving incident and may therefore be irrelevant in assessing impairment [142]. Moreover, a blood sample is required by the law for DUID investigations in several countries, including Finland [7].

The process for roadside and laboratory tests in association with suspected DUI cases is illustrated in Figure 6.

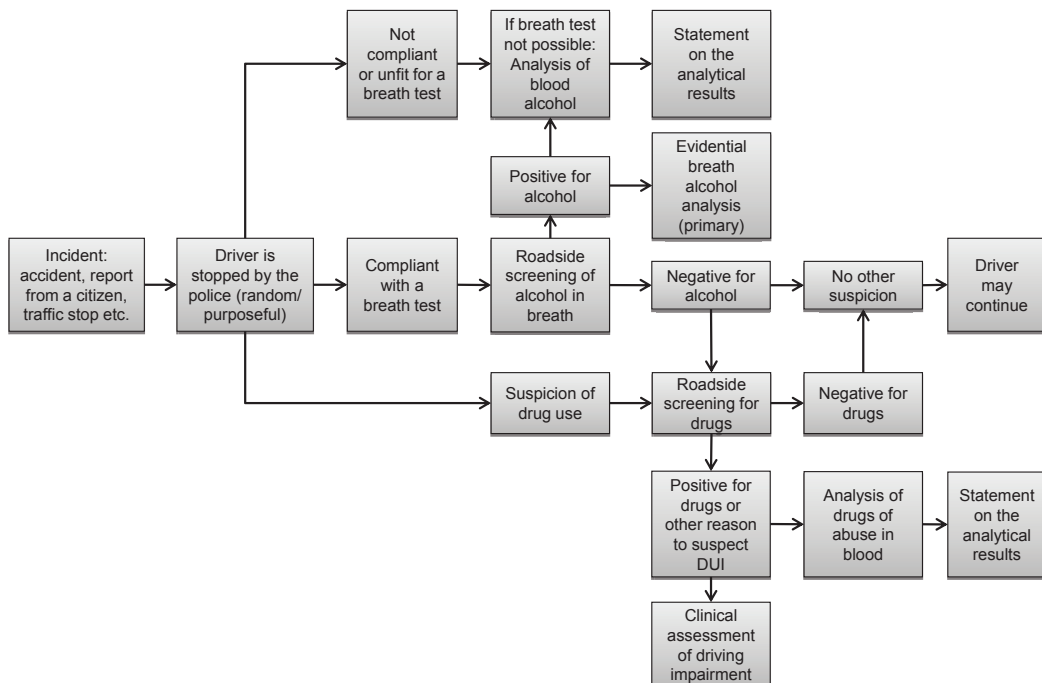


Figure 6. Process chart of the initiation of a DUI investigation.

In Finland, the extent of the drug screening that is performed on a particular blood sample depends on the severity of the preliminary charges which are determined by the police officer in charge of the preliminary investigation. A prosecutor only comes to the picture after the preliminary investigation is finished. In DUI cases, the preliminary prosecution – and later the actual charges – can either be “driving while intoxicated” or “driving while seriously intoxicated” depending on the circumstances, clinical assessment of impairment and the arresting police officer’s evaluation of the situation. In alcohol cases the division into the two categories is simple: if the roadside breath test shows an alcohol concentration between 0.50 and 1.19 g/kg the offence is ordinary DUI. If the alcohol concentration is equal or above 1.20 g/kg the offence is graded as aggravated (“driving while seriously intoxicated”). The charges may change later if the results of the analysis of the blood sample differ from the screening results.

In drug cases, the categorisation is more based on the observations of the arresting police officer and other data on the incident. The final charges (normal vs. aggravated) will be determined at the end of the preliminary investigation.

2.5.2 Impairment testing

One of the means available to study driving impairment caused by drugs is to examine competence tests performed when drivers are apprehended for DUI. The standardised clinical examination in Finland consists of 15 tests of motor, vestibular, and mental impairment, such as walking on a straight line, finger-finger test, speech, pupil size and reaction to light, and mood. In other countries, several different kinds of impairment and sobriety tests are used. All of these tests share the same limitation in that sedative induced impairment is relatively easy to detect whereas the effects of stimulant use often remain undetected. Even sedative use can sometimes be missed: In Norway, many of the drivers examined passed the tests even with considerable blood concentrations of zopiclone or alcohol [143].

The test used in Victoria, Australia has shown reasonable success in detecting impairment following recent use of cannabis [144] but is less successful with amphetamines [3]. The roadside impairment test used in Norway shows a positive concentration-impairment relationship for amphetamines [145]. The German procedure is most effective in detecting impairment in opiate users but its performance with other classes of drugs was found to be adequate for screening purposes [146].

2.5.3 Recidivism and attitudes towards DUI

According to earlier studies from Sweden and Finland, recidivism is very common among drivers arrested for DUI [147,148]. This is especially prominent in drivers using drugs when compared to those driving under the influence of alcohol only [148]. Depending on the study, a third [148] or even nearly half [147] of the drivers re-offended after the first arrest. In the Finnish study, the use of stimulants in particular was found to be associated with an increased incidence of early re-arrest [148].

The attitude of the driver towards DUI has been suggested to differ for those driving under the influence of drugs versus those driving under the influence of alcohol. Drivers under the influence of alcohol often regret their behaviour afterwards while people driving under the influence of drugs already consider themselves to be outside the law as a result of using illegal drugs and show less regret for the DUI offence [149]. As compared to drug use itself, the illegality of the driving behaviour is in their opinion considered a minor offence [150]. Moreover, those

driving under the influence of either drugs or alcohol consider the risk of being caught to be low [150].

Those committing DUID may also assume that the drug use will not increase their risk of an accident but perhaps even improve their driving skills [149,150]. Drivers have been shown to be unable to recognise their driving impairment caused by drugs [151]. Some may get part of the thrill of drug use from driving while high [150]. Avoiding driving under the influence of drugs would require significant effort by users and likely cause considerable inconvenience (such as delays in achieving their next “high”). In addition, the fact that they are illicit drug users means that they are already failing to regulate their behaviour. These factors combine to make DUID very difficult to prevent [149].

2.5.4 Effects of drugs on driving performance

All drugs that affect the CNS – whether prescribed or illegal drugs – may cause driving impairment by affecting crucial driving skills such as reaction time, judgment and processing simultaneous tasks [152]. Many of the effects are dose-dependent even in experienced users [153] although for some of the effects tolerance may develop with time.

Sedatives, such as cannabinoids, opioids, benzodiazepines and *gamma*-hydroxybutyric acid (GHB) can affect ability to concentrate and can cause drowsiness, lack of coordination, altered perceptions, memory impairment, and slower reaction time, all of which all are detrimental for driving [154,155,156,157]. Sometimes the effects of the drug (e.g. cannabis) cannot be identified by the driver so that they may consider themselves competent while actually being impaired [158]. Older individuals are in general more sensitive to the effects of sedatives, such as benzodiazepines, and the effects also last longer [159].

Stimulants, such as amphetamines, cocaine, and synthetic cathinones, can increase the driver’s tendency to take unnecessary risks while at the same time affect concentration and impair vision [145,160,161,162]. At least in low doses, however, stimulants may improve psychomotor performance for example by enhancing alertness [163]. Combining drugs that have similar effects on the CNS may cause synergistic effects so that the overall effect is greater than the sum of the individual effects [157]. Under the influence of amphetamine-like stimulants, users often do not show any signs of impairment in the test systems available for routine screening, although impairment with such drugs is detected in more sophisticated laboratory systems; this can make prosecution in such cases challenging [164].

Opportunities to study the pharmacology and toxicology of NPS are limited for obvious ethical reasons. The vast majority of the publications about NPS describe analytical determination of the substances or case reports. There is very little data available so far about prevalence of NPS among drivers or how the NPS actually affect driving performance. However, when taking NPS, the user is likely seeking effects similar to those of the more traditional abused drugs. Thus the pharmacology of NPS is likely to have some similarity to traditional abused drugs and adverse effects on psychomotor performance are likely to be similar. Although this is a reasonable assumption, it is only an assumption and important differences may exist that could be revealed in driving simulation studies with volunteer subjects; such studies (administering unapproved drugs with no safety data to healthy volunteers), however, would be unethical and are unlikely to be performed. The typical neurological and psychological effects of synthetic cathinones, such as agitation, dizziness, panic, paranoia and hallucinations, can have major detrimental impact on driving performance [78].

Some reports describing the driving impairment caused by NPS have been published in recent years although they are reports of individual cases of DUID rather than planned trials. In Seattle, USA, a driver apprehended for poor driving showed the following symptoms after being apprehended: slurred speech, bloodshot watery eyes, dilated pupils, involuntary muscle movements and an elevated pulse and blood pressure. In toxicological analysis of the driver's blood, α -PVP (0.063 m/L), methylene (0.0061 mg/L) and ethylene (positive) were found [165]. On the basis of the clinical examination, he was judged by the examining physician to have been under the influence of CNS stimulants and was placed under arrest. This is a rare case of the use of synthetic cathinones with no other psychoactive substances present and it thus provides important information about the possible effects of a particular class of NPS on psychomotor performance.

Another study from the US (Ohio) reports on seven drivers positive for MDPV over a period of about 2 years. Some of the subjects had other psychoactive substances present as well, but rarely in pharmacologically relevant concentrations [76]. An interesting finding in this particular study was that blood cathinone concentrations did not appear to correlate with the observed driving impairment. Some relatively low concentrations were associated with major behavioural changes. The authors of that study suggested that this might be related to the dysphoria ("crash") commonly experienced after a prolonged stimulant high [76,166].

In a German study, the toxicology results from six DUID cases were presented together with information about signs of impairment. Many of the drivers showed concomitant use of other drugs together with the studied phenethylamines but at least in some cases the observed impairment was suggested to result from the use of MDPV and desoxypipradrol [78].

There are more data available on driving under the influence of synthetic cannabinoids. A study from Germany described eight individuals examined toxicologically for DUI of synthetic cannabinoids. Only one of the subjects had another psychoactive substance, namely alcohol, in their blood and all showed clear signs of impairment [167].

In Norway, 2.2% of 726 drivers apprehended for DUI and analysed for synthetic cannabinoids were found to be positive for at least one of them. However, the concomitant use of other drugs made it impossible for the researchers to evaluate the role of synthetic cannabinoids in the observed driving impairment of the subjects [168]. In Pennsylvania, USA, synthetic cannabinoids were found to have caused cognitive and psychomotor impairment to 12 drivers apprehended for unsecure driving [169].

Phenazepam has also been detected in DUID cases in the past few years but there is no direct assessment of its impact on driving. However, being a benzodiazepine, the effects of phenazepam on driving skills are likely to resemble the effects of diazepam although its long elimination half-life and greater potency suggest that the induced impairment is also likely to be stronger and/or last longer [153]. In addition, due to the long elimination half-life of phenazepam (approximately 60 hours), the risk of unintentional intoxication by rapid re-dosing may be an issue [90].

The only information on driving under the influence of pregabalin comes from two driving simulator studies with healthy volunteers [136,137]. In one of the studies, the administered dose resembled the typical therapeutic dose of 150-600 mg [170] and it caused minor, transient impairment on certain psychomotor skills [136]. In the other study, the administered dose was low (75 mg on two consecutive days) and it mainly caused sleepiness in the tested volunteers [137]. The reported side effects of pregabalin, such as dizziness, sleepiness and decreased concentration [170] suggest that the drug may cause impairment, especially taken in high concentrations [171].

2.6 NPS in post-mortem cases

In Finland, about 12000 forensic autopsies are performed every year. In about 6000 of these cases post-mortem toxicology is requested; as a result, toxicology is performed on approximately 13% of all deaths in Finland. The samples are analysed for a wide variety of drugs and approximately 300 cases result in drug findings annually. The selection of analysed drugs and medicines is frequently updated when new substances emerge. Toxicologists at the forensic laboratory performing the analyses actively search for information on new substances. In addition to the scientific literature, potential sources of useful information are the data provided by the Finnish Customs and Police, the European early warning system (EWS) and various international toxicology meetings.

In various countries over the years, probably the greatest number of deaths in which NPS were detected included mephedrone [172,173,174,175,176,31] or mephedrone together with other synthetic cathinones. In addition, methedrone, butylone, methylone, buphedrone *para*-methoxymethylamphetamine (PMMA) and especially MDPV have been frequently detected in post-mortem cases [117,177,178,179,180,181,182, Table 2]. However, the post-mortem investigation process varies significantly in different countries; the range of drugs included in the screening procedures varies as does the nature of the samples analysed (Table 2).

To date, the only population based study on drug-related deaths (DRD) involving NPS was published in 2014 by a Scottish group [117]. In their study, 36 cases with a NPS findings were recorded in 2012 and in 23 of these, NPS were implicated in the actual cause of death [117].

The broad variety of NPS and the constantly changing selection of drugs to be analysed have created new challenges for post-mortem toxicology. Additionally, the lack of reference standards or their high cost may in some cases greatly delay the incorporation of new substances into the screening program. These challenges have been addressed with novel analysis techniques such as HRMS [68].

In countries, such as Finland, where a considerable proportion of deaths undergo a medico-legal cause-of-death investigation and toxicological analysis, recognizing the arrival of new drugs is easier than in countries with a lower rate of post-mortem toxicology. In Finland, nearly all drug-related death cases are likely to undergo post-mortem toxicology so the possibilities of detecting new trends in drug use patterns are high. Only after sufficient information on blood concentrations, clinical symptoms and drug use profiles of the NPS has been accumulated, can the toxicological information from post-mortem investigations be used in interpretation of the cases.

2.7 Banning of NPS

In Finland, NPS are regulated by the Narcotics Act [183]. The legal definition of a narcotic or psychotropic agent is set by the Finnish Narcotic Act and all banned substances are listed in the Government Decree (543/2008) [184]. Once a substance has been evaluated and is to be banned, it is added to the list in Government Decree (543/2008) [185].

The Finnish Medicines Agency, Fimea, proposes to the Ministry of Social Affairs and Health which new substances should be classified as narcotic drugs. Before being scheduled as narcotics, NPS are banned from the consumer market and listed in a separate Decree (1130/2014) [186].

The listing of an NPS as a substance banned from the consumer market is conducted after evaluating its pharmacological properties. Unlike with narcotic drugs, possession and use of

substances banned from the consumer market is not criminalised. These substances are defined as those “used for intoxicating purposes that might be a danger to health and that have been decided to be made subject to control in accordance with the EU Council Decision or are positional isomers of such a substance and that are neither medicines nor narcotic drugs” [186].

The procedure for evaluating substances proposed for listing as narcotic drugs is more thorough than that required for banning from the consumer market. In the process of scheduling NPS as narcotics, various characteristics of the substance or product are evaluated, including the pharmacological/toxicological properties, potential uses, exposure levels (concentration/duration), information about potential users and risks associated with the substance. In most cases the process takes about 1-6 months and there is not always much evidence-based information available, which is typically the case for the NPS.

In Finland prior to June 2011, an NPS could not be added to Government Decree (543/2008) and brought under control of the Narcotics Act until similar action had been taken by the EU or UN. However, in June 2011 the Narcotics Act was modified to allow the banning of an NPS in Finland without prior EU or UN action. This change should enable more rapid regulation, particularly in those cases in which a particular NPS presents a problem in Finland but not in other countries.

When evaluated retrospectively, banning decisions have been found to be mostly correct [187]. Banning of potentially beneficial substances that may have medicinal use must be avoided while meeting the primary goal of reducing harm by keeping dangerous drugs out of the marketplace [188].

3 AIMS OF THE STUDY

The aim of this study was to assess the prevalence, blood concentrations and significance of selected new abused drugs in DUID cases and in post-mortem investigations in Finland and to characterise the toxicological profile of users (I–V).

The more specific aims of the thesis were:

- To determine the prevalence and blood concentrations of 3,4-methylenedioxypyrovalerone (MDPV) and to investigate the concomitant use of other drugs in MDPV-positive DUID cases as well as the geographical distribution of the cases in Finland in a one year period between 28 August 2009 and the end of August 2010 (I).
- To look at the same factors and issues in MDPV-positive DUID cases over a longer time span between August 2009 and December 2012 with additional information from post-mortem cases in the same time period (V).
- To use the data from psycho-physical assessments conducted after arrest to evaluate the significance of the presence of MDPV (I).
- To investigate the police confiscations of NPS in order to gain information on the prevalence of MDPV (I).
- To determine the differences in blood concentrations, user profiles and concomitant use of other drugs in DUID and post-mortem cases before and after MDPV was banned, and to determine whether the impact on court decisions of finding MDPV in DUID cases changed after it was banned (V).
- To determine the prevalence, blood concentrations and significance of phenazepam in drivers apprehended for DUID and in post-mortem blood samples in Finland and to assess the geographical distribution of the phenazepam-positive DUID cases in a one year period between July 1, 2010 and June 30, 2011 and to look at the police confiscations of the drug (II).
- To determine the prevalence, blood concentrations and significance of desoxypipradrol (2-DPMP) in drivers apprehended for DUID and in post-mortem cases in Finland between October 2010 and May 2012 and to look at the police confiscations of the drug (III).
- To determine the blood concentrations of pregabalin in blood from DUID cases in Finland in a one year period in 2012, to assess the concomitant use of other drugs by drivers positive for pregabalin, and to re-evaluate the abuse potential of this drug formerly considered to be free of such problems, even when used by drug addicts (IV).

4 MATERIAL AND METHODS

The main focus of this thesis was the toxicology data from DUID investigations and post-mortem studies in Finland. Post-mortem and DUID toxicology data were compared with background data on the victims/culprits obtained from public records. Data on police confiscations of the studied drugs provided by the National Bureau of Investigation in Finland were also examined. The study period was 2009–2012. Further breakdown of the study periods, materials and registers used and number of cases studied in each of the five studies presented in the original publications I–V are illustrated in Figure 7.

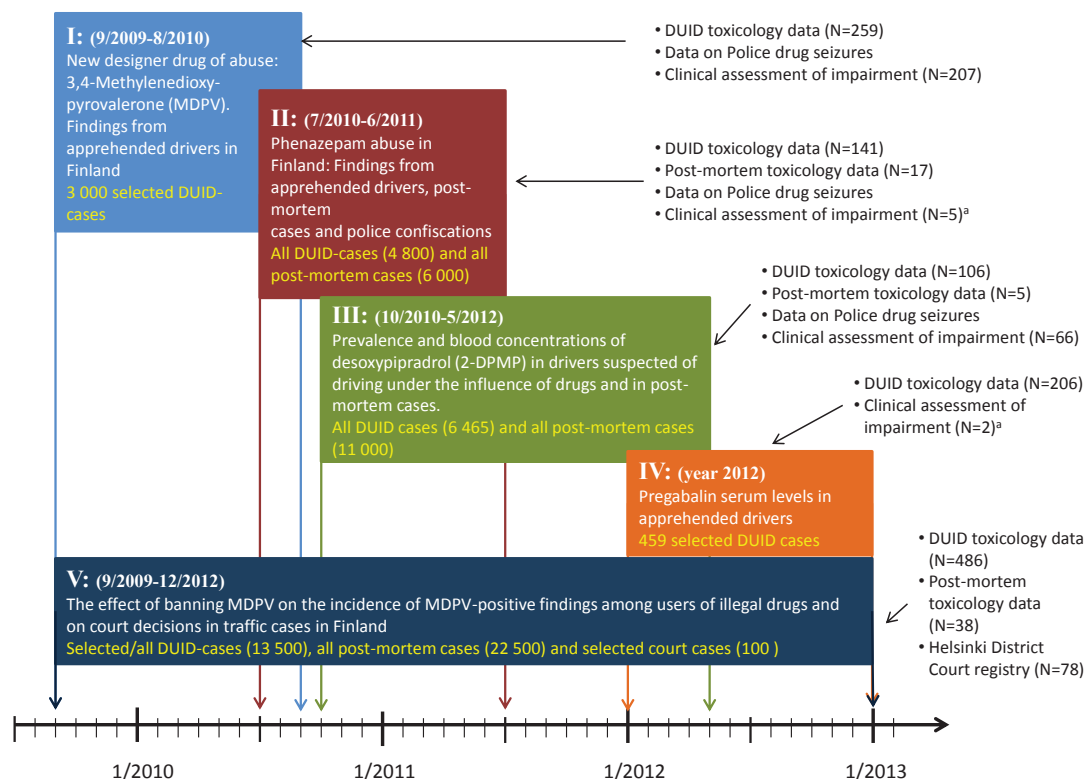


Figure 7. Timeline of the individual studies included in the thesis (I–V). The numbers in the boxes represent the size of the studied population/material. The numbers (N) on the right of the boxes are the number of cases studied in each part of the thesis (e.g. number of MDPV-positive samples in the studied population, or the number of separate court cases that included the 100 randomly selected DUID cases).
^aOnly the clinical examinations of single-drug cases were examined.

Detailed descriptions of the materials, such as reagents and reference material used in specific parts of the study as well as method information and validation data are presented in the original publications (I–V).

4.1 DUI investigations

In the four-year study period between 2009 and 2012, the number of driving cases for which the police requested toxicological analysis ranged between 3863 and 4569 per year (Police statistics). All toxicological analyses for DUID investigations were performed in a single laboratory. The drug screening and confirmation analysis procedures were designed by the Forensic laboratory of the National Bureau of Investigation in Finland in co-operation with the Office of the Prosecutor General. During the time period studied in this thesis the analytical procedure in DUI cases was as follows:

a. Driving while intoxicated –cases:

A comprehensive screening for abused drugs and medicines including amphetamines, cannabis, cocaine, benzodiazepines and opioids (also buprenorphine) was performed by immunological methods. Additionally, comprehensive screening (liquid chromatography – tandem mass spectrometry (LC-MS/MS) was performed for every sample to complement the immunological screening. The LC-MS/MS enabled high sensitivity screening for NPS in every sample, while the sensitivity of immunological methods for different NPS is poor. All positive findings were confirmed by LC-MS/MS.

b. Driving while seriously intoxicated –cases:

In addition to the comprehensive screening (see above), screening for a selection of further illegal and medicinal drugs was conducted by means of gas chromatography – mass spectrometry (GC-MS) and LC-MS/MS.

Blood and serum samples were taken at the nearest health centre as soon as possible after the driver was apprehended. In cases of suspected heroin use, urine was also collected for the analysis of 6-acetylmorphone. In Finland, use of heroin has been extremely rare ever since the heroin shortage in Europe in 2001, which is why urine was only seldom collected in the study period (in less than 10% of the cases). The police officers sent the blood and urine samples to the laboratory together with background information on the case. The police were instructed to store the samples in a refrigerator if it was not possible to mail them immediately.

All samples were delivered to the laboratory without identifying the driver. Samples were coded with 8 digit numbers. Thus it was not necessary to de-identify the samples before the study. Age and gender as well as time of the incident and time of blood sampling were recorded in the laboratory electronic record-keeping system.

4.1.1 Road-side testing of DUID suspects

In most cases, a driver is apprehended on suspicion of DUID because somebody reported them to the Police for unsafe driving or observed use of drugs or alcohol before driving. In some cases a driver may be apprehended because of a driving error that was observed by the Police. It is not common that suspicion of DUID would arise from a routine traffic stop check.

The device most commonly used in road-side screening for drugs in the studied cases was DrugWipe 6s[®] by Securetec. The device consists of a miniaturised immunoassay that screens for amphetamines, cannabis, benzodiazepines, cocaine and opiates in oral fluid. The device is operated by wiping the tongue or the inside of the cheek of the suspect. After completing a simple processing procedure, the results are displayed after an 8 minute waiting period.

Regardless of the results of the roadside drug screening test, the driver could be held for further examination if there was reason to believe that they may have been under the influence of drugs. The results of the road-side screening test assist decision making by the arresting police officer but they do not have evidentiary status so cannot be used in court.

4.1.2 Clinical assessment of driving impairment

Shortly after being caught, drivers suspected of DUID were subjected to a clinical assessment of impairment. This standardised examination was performed by a physician as per the guidelines provided by the Finnish Ministry of Social Affairs and Health. The examination covered a total of 15 tests of motor, vestibular, and mental impairment. More details of the examination are given in paper III. The results of the clinical assessment – if performed – were used in the DUID investigation process in combination with the laboratory results and witness statements.

4.1.3 Analytical methods

The analytical methods were validated according to the guidelines of German standard specification DIN 32645 [189] and other guidelines for method validation in the forensic field [190,191]. Details on method validations are presented in the original publications (I-IV) and in Table 3. The methods did not remain the same throughout the study period. They were constantly developed to better meet the challenges of a massive sample load and strict timeframes of the DUID investigations. Moreover, new substances were regularly added to the methods when there was reason to believe that they were being used by Finnish drug users. However, the methods for the analysis of the substances in this thesis remained the same throughout the study periods (Fig. 7), except for the MDPV method in DUID cases; at the beginning of the study period the MDPV methodology was a qualitative assay, but later, with the availability of commercial reference material, was further developed to be a fully validated quantitative method.

Sample preparation methods included solid phase extraction (SPE), protein precipitation (PP) with acetonitrile or liquid-liquid extraction (LLE). The method used for particular substances as well as the analytical methods are listed in Table 3.

The drug screening procedure consisted of an immunological screening followed by a multi-analyte LC-MS/MS screening using multiple reaction monitoring (MRM). All samples underwent both screening procedures - a negative result from the immunoassay did not rule out LC-MS/MS screening. Most of the drugs frequently encountered were quantified in the LC-MS/MS screen but some required an extra quantification step. However, apart from pregabalin, all analytes studied in this thesis were quantified directly from the screening run. At the time of the study, pregabalin was only analysed when requested or when there was other reasons to believe that the driver had taken pregabalin (N=459, 12% of all DUID samples in the study period), and consequently the drug was quantified by a separate method (Table 3).

Table 3. Details of the analytical methods used for the analysis of the studied substances in the serum samples of DUID cases.

	Sample preparation	Method	Calibration range (mg/L)	LLOQ ^a (mg/L)	Q1-Q3 ^b (m/z)	ISTD ^c
MDPV	SPE	LC-MS/MS	0.010 – 0.500	0.003	276→126 276→135	MDEA-d5 ^d
Phenazepam	SPE	LC-MS/MS	0.003 – 0.100	0.0014	351→206 351→185	Diazepam-d5
2-DPMP	SPE	LC-MS/MS	0.003 – 0.500	0.003	252→91 252→167	MDPV-d8
Pregabalin	PP	LC-MS/MS	0.68 – 20.00	0.22	160→142	2-amino-3-cyclohexyl-1-propanol

^alower limit of quantification, ^bmonitored transitions in the MS/MS, ^cinternal standard,

^d3,4-methylenedioxy-*N*-ethylamphetamine, deuterated,

4.2 Post-mortem investigations

All post-mortem samples in this study were analysed in one laboratory, namely in the toxicology laboratory of the Department of Forensic Medicine in the University of Helsinki. The analytical procedure for post-mortem samples is illustrated in Table 4.

Table 4. The analytical screening procedure of post-mortem samples applicable to this study.

Matrix	Type of analysis	Equipment	Target compounds	Reference
urine	screening	LC-TOFMS	~700 drugs	192,193
blood	quantitative	GC-NPD ^a	acidic/neutral drugs	194
		GC-ECD ^b	benzodiazepines	195
		GC-NPD	basic drugs	196
blood/urine	confirmations	CG-MS and LC-MS/MS		
blood/urine	quantitative	HP-GC-FID ^c	alcohol	

^anitrogen-phosphorus detector, ^belectron capture detection, ^cheadspace gas chromatography with flame ionisation detection

For all of the studied substances in post-mortem cases, sample preparation included LLE followed by derivatisation of MDPV and 2-DPMP with heptafluorobutyric anhydride (HFBA) and phenazepam with *N*-tert-Butyldimethylsilyl-*N*-methyltrifluoroacetamide + 1 % tert-butyldimethylchlorosilane (MTBSTFA + 1% TBDMSCI). The analytical parameters for the studied substances are listed in Table 5. All post-mortem blood results reported in this study originate from the analysis of femoral blood.

Table 5. Details of the analytical methods for the studied substances in post-mortem blood samples.

	Confirmation method	Calibration range (mg/L)	LLOQ (mg/L)	Target ion (<i>m/z</i>)	Qualifier ions (<i>m/z</i>)	ISTD
MDPV	EI ^a -GC-MS	0.02–2.00	0.02	126	149,121	MDPV-d8
Phenazepam	CI ^b -GC-MS	0.005–0.40	0.005	312	314	Temazepam-d5
2-DPMP	EI-GC-MS	0.01–5.00	0.01	280	165,226	MDMA-d5

^aelectron ionisation, ^bchemical ionisation

4.3 Police confiscations of NPS

Information on the number of samples of the studied substances in material confiscated during the studied time period was received from the Forensic Laboratory of the National Bureau of Investigations when requested. One confiscated sample can mean 2000 tablets or 1 gram of a substance so the numbers given for different batches are not directly comparable but do provide a very rough estimate of the relative amounts of the drugs seized.

4.4 Court sentences in DUID cases

The District Court in Helsinki is one of 27 in Finland. It serves a population of over 600 000 inhabitants in the Capital of Finland and handles around 1800 DUID cases every year.

After receiving research permission from the Finnish Legal Register Centre, DUID case numbers and the numbers assigned to police reports of offences were linked and a database search was conducted. From the database, 100 court cases positive for MDPV and located in Helsinki were randomly picked so that about half of the cases were from the time before MDPV was banned and the other half from the time after it. The randomness of the sampling was ensured in that the cases were picked from a list of numbers of MDPV-positive samples. There was no other data on the cases in the list besides the numbers so that the person picking the cases did not know any details about the cases. Thus, the specifics of the cases, such as MDPV concentration, did not impact the selection of cases. Due to the relatively large sample size (100 cases) and the random selection of court cases that were included in the study, the sample can be assumed to well represent the population of MDPV-positive apprehended drivers. Between 2009 and 2012 there were 211 DUID cases positive for MDPV in Helsinki Police Department, thus the number of court cases selected for the study covers nearly half of the cases in the study period.

The selected cases were examined and scanned at the archive of the District Court of Helsinki. Age and gender as well as the number of charges and other details of the cases were recorded. Particular consideration was given to whether MDPV was mentioned in the court documents and reasoning.

4.5 Statistical methods

All statistical analyses were performed using IBM SPSS software (versions 18.0–20.0). For the comparisons, the Pearson Chi-Square test or the Fisher's exact test were used for dichotomous variables, the independent-samples T test for continuous variables, and the Mann-Whitney, Spearman's rho or the Kruskal-Wallis test for non-parametric variables.

4.6 Research ethics

Analytical data, background information on the cases and information on the DUID incident without any personal identifiers could be used for this register-based research without approval of the Ethical Committee. For the study that included court documents, research permission from the Finnish Legal Register Centre was obtained. The cases were coded and registered into a database according to the guidelines of the Finnish Legal Register Centre.

5 RESULTS AND DISCUSSION

In Finland, the yearly number of DUI investigations in which a blood sample is collected is over 12 500. In approximately 4000 cases there is a drug request involved either alone or in addition to a request of alcohol analysis. In this thesis, the serum concentrations associated with abuse of selected NPS were studied; for some of the substances this is the first time such data has been obtained. As can be seen from Table 2, previous data on blood concentrations associated with

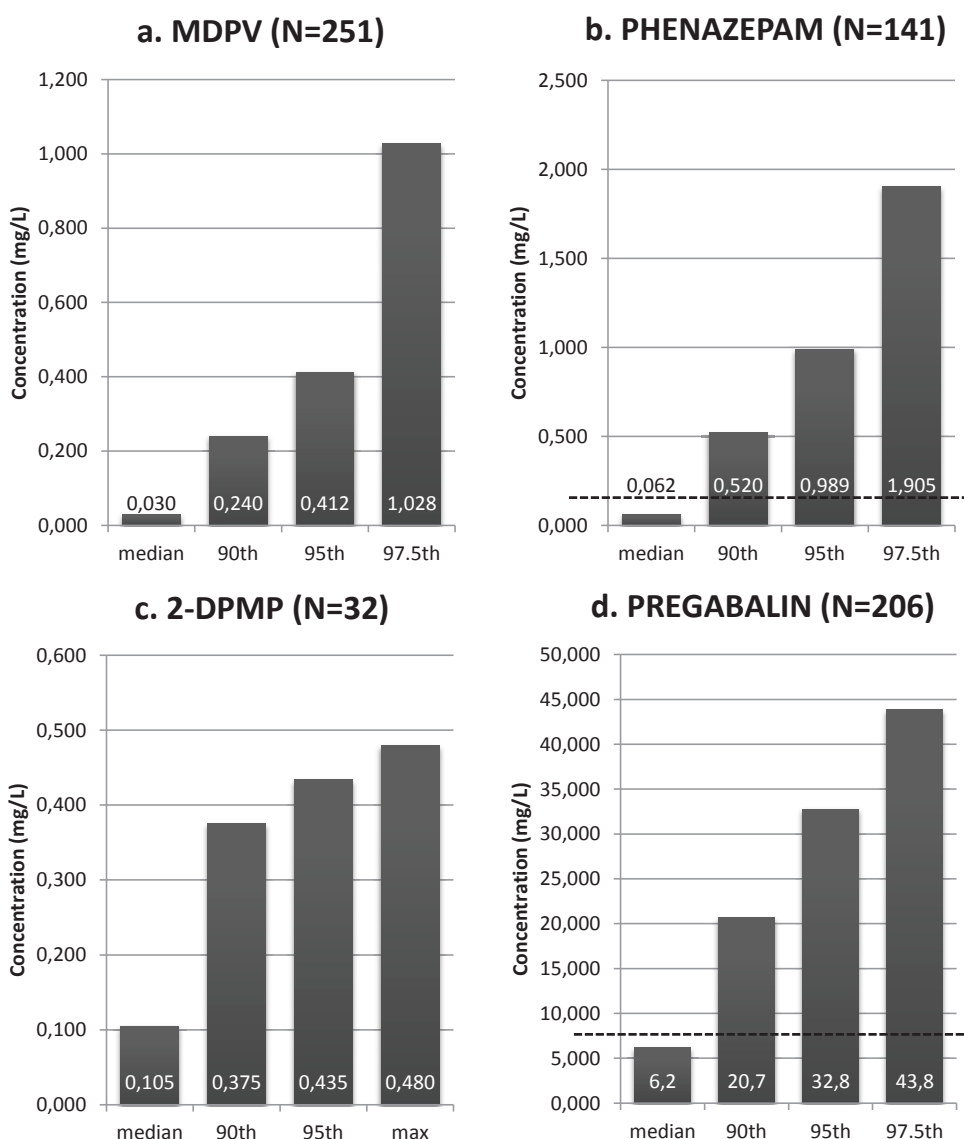


Figure 8. Concentration medians and percentile values for the studied substances (II–V). The dashed line (b. and d.) illustrates the upper limit of the therapeutic range. For 2-DPMP the maximum concentration is given instead of the 97.5th percentile since the number of cases with a quantitative result was too small for the calculation of the 97.5th percentile.

abuse of these substances are for the most part anecdotal, based upon reports of single cases. Fig. 8 shows the results obtained from the individual studies in this theses in terms of median concentrations and the 90, 95 and 97.5 percentiles of the studied substances in serum. Since the number of cases positive for the studied substances in the study periods was not always the same as the number of cases for which quantitative results were obtained, the latter is given in Fig. 8 next to the name of the substance.

As seen in Fig.8., the differences between median concentrations and the 97.5th percentile values were extremely large for all of the studied substances but especially for MDPV indicating that there were some very high concentrations included in the data. The fact that some individuals were able to drive, or at least thought they could drive, with such high concentrations of MDPV in their system is probably due to the development of tolerance to the pharmacological effects of the stimulant [151]. Similarly, in Sweden, blood amphetamine concentrations as high as 5–17 mg/L have been reported in drivers [197] even though such concentrations are normally associated with fatal intoxications.

In general, the use of many medicinal drugs is concentrated in older age groups whereas drug abuse is more prevalent among younger individuals. The age and gender distribution of the studied substances in DUID cases and the age distribution in all Finnish DUI cases in 2012 is illustrated in Fig. 9.

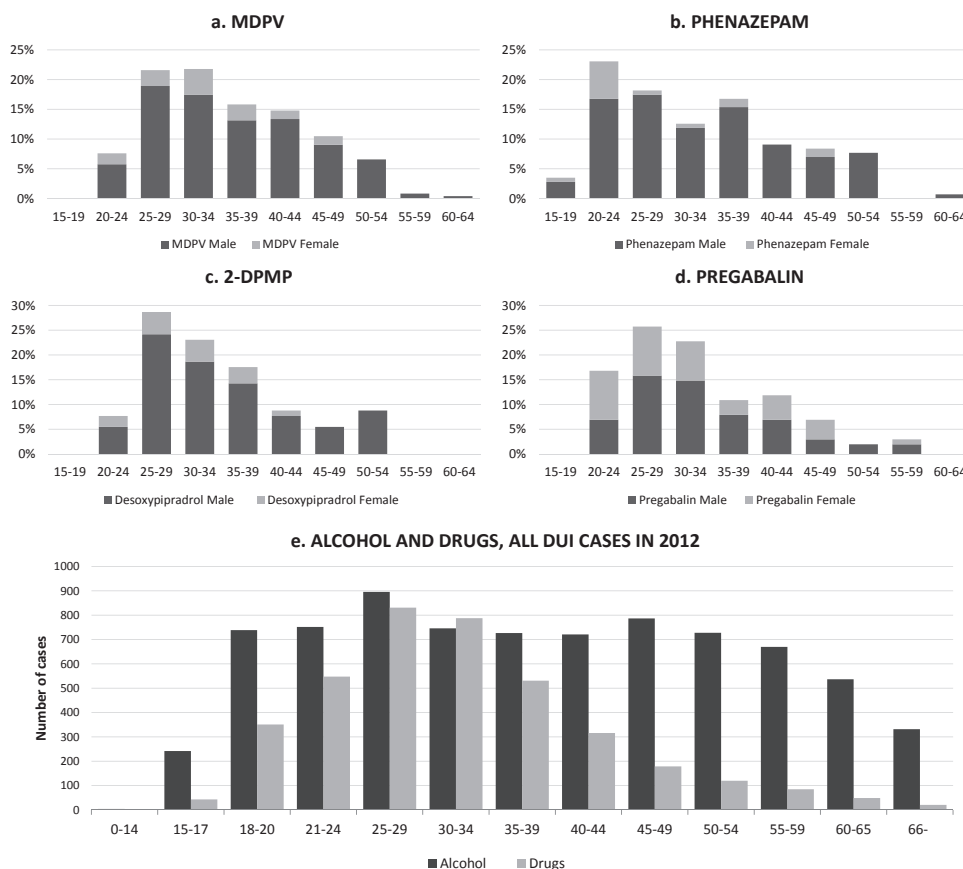


Figure 9. Age and gender distribution of MDPV, phenazepam, 2-DPMP and pregabalin in the study periods and of all drivers positive for alcohol or drugs in 2009–2012. (a–d: II–V; e: Police statistics)

As seen in Fig. 9., females were proportionally much more represented in pregabalin-positive cases (41%) than in cases positive for other studied substances (11–15%) ($p < 0.001$) [VI]. Moreover, there were proportionally more women in younger age groups (under 35 years) of all studied substances than in older age groups [VI]. None of the substances were frequently encountered in individuals aged 60 or older. The mean ages of drivers positive for each of the studied substances are illustrated in table 6.

Table 6. Mean ages of the drivers positive for the studied substances and the statistical significances of the age differences between genders (II–V).

	Mean age		Significance of the age difference	Ref
	males	females		
MDPV	36	33	$p < 0.012$, t test	V
Phenazepam	34	27	n.s.	II
2-DPMP	35	31	n.s.	III
Pregabalin	33	32	n.s.	VI

The frequency distributions of the studied substances in different age groups as illustrated in Fig 9. resemble that of drug users in general in that the highest numbers of cases are in younger age groups except for phenazepam, the profile of which is two-peaked resembling the profile of drivers positive for alcohol (Fig. 9e). An explanation for the different profile for phenazepam could not be deduced from the study material nor from earlier literature. In terms of age differences in drug use patterns, previous studies have shown that whilst young individuals may experience the rewarding properties of drugs more strongly than older individuals, their sensitivity towards the adverse effects of drugs may be attenuated, which may increase the probability of continuing the use after the first experiences [198].

5.1 MDPV

5.1.1 MDPV-positive DUID cases in the early stage of its appearance in Finland

In this thesis, MDPV was studied twice. In the first part (I), MDPV-positive cases in the DUID population were assessed at an early stage of its appearance in the illegal drug market. At the time, awareness of NPS in general was not widespread and many health care professionals did not know that they existed. MDPV was the first designer drug that gained widespread publicity in Finland, largely because it was connected to some brutal crimes, including homicides.

In 2009 and 2010, MDPV was frequently detected in DUID suspects in Finland. Nearly 6% ($N = 259$) of all positive DUID cases in the one-year study period were positive for MDPV (I). The main contribution of study I to toxicological knowledge was the first publication of serum concentrations associated with abuse of MDPV. The large number of cases in this study provided exceptional material for that purpose. At the beginning of the study period, only a qualitative assay for MDPV was available, thus quantitative results are only available for a subgroup of the samples. The concentration of MDPV ranged between 0.016 mg/L and 8.4 mg/L (median 0.22 mg/L) (I). The median concentration is markedly higher than reported in a very recent

Australian study on 33 MDPV-positive drivers (0.05 mg/kg) [81]. When compared to a Swedish study with over 26 000 driving cases, the median MDPV concentration in the present study was higher than that of MDMA (0.10 mg/L) but lower than the median amphetamine concentration (0.70 mg/L) in the Swedish material [199].

Most of the MDPV-positive drivers were male (87%) and the majority was aged between 25 and 44 years (76%) (I).

In most cases (96.9%), MDPV was found together with other potentially impairing psychoactive substances; most often amphetamine (80%) or benzodiazepines (67%) or both (54%) (I). It appeared that the blood levels of sedatives used together with MDPV were relatively low when compared to DUID cases without MDPV use. In contrast, concentrations of other stimulants used together with MDPV were high. Alcohol was only rarely found in MDPV-positive cases, and mainly in very low concentrations (I).

5.1.2 DUID and post-mortem cases positive for MDPV over a 3½ year period

In the second study related to MDPV (V), a longer time period (about three and a half years) was investigated as well as the post-mortem cases positive for MDPV in the same time period. In this study, the number of MDPV-positive DUID cases was 486 and the number of MDPV-positive autopsy cases was 38. The median serum concentration of MDPV in DUID cases was 0.030 mg/L and in post-mortem cases 0.12 mg/L (V).

In the study period, 86% and 79% of the cases were males in DUID and post-mortem cases, respectively. In DUID cases, women were significantly younger (mean 33.2 years) than men (36.1 years). In post-mortem cases the age difference between the two genders was even more prominent (women 24.3 vs. men 35.9 years). Additionally, the average age reported for MDPV-positive female fatalities was considerably lower (24.3 years) than the average age of all fatal poisonings of users of illicit drugs (both genders, all drugs) in Finland in 2012 (33.2 years) [11] (V).

In the majority of cases, MDPV was detected together with various other drugs. It was the only drug found in only one post-mortem investigation and in only 4% (N=20) of the MDPV-positive DUID cases. In 22% of the MDPV-positive DUID and 42% of the MDPV-positive post-mortem cases, MDPV was the only stimulant detected. In MDPV-positive DUID cases, males had an average of 4.2 other drugs present besides MDPV whereas the number in females was 3.6 ($p=0.018$). In MDPV-positive post-mortem cases the mean number of other drugs was 4.5. Amphetamine (DUID 75%, post-mortem 58%), benzodiazepines (DUID 74%, post-mortem 71%), cannabis (DUID 28%, post-mortem 24%) and opioids (DUID 13%, post-mortem 68%) were frequently encountered in both MDPV-positive DUID and MDPV-positive post-mortem populations. In DUID cases, the number of other drugs used together with MDPV decreased slightly with age ($p<0.001$ Spearman's rho) (V). MDPV was often encountered together with other NPS, e.g. 2-DPMP, methylone and synthetic cannabinoids. Some of these findings may be due to the substances being sold as mixtures (V).

It is quite remarkable that alcohol was present only in 1.6% of the MDPV-positive DUID cases (N=8). However, alcohol was not analysed in every sample, but only when the police had requested it, meaning that in some cases alcohol may have been present but not analysed. In post-mortem cases, however, alcohol was analysed in every sample and the percentage of alcohol-positive MDPV-positive cases, 21% (N=8), was closer to the rate reported for fatal

poisonings of users of illicit drugs in Finland in general (36.4%) [11]. Nevertheless, even if all MDPV-positive DUID samples had been screened for alcohol, given that the drug use profile was quite different between MDPV-positive DUID and MDPV-positive post-mortem cases in respect to CNS sedatives such as opioids (V), it is unlikely that alcohol use would be the same in the two populations.

Of the 38 deaths in the study period involving MDPV, 28 were determined by the forensic pathologist to be fatal poisonings. Of these, two were poisonings by MDPV only and the other 14 were multi-drug poisonings with MDPV together with other drugs. The remaining 12 poisonings included poisonings with propranolol, doxepin, opioids and carbon monoxide and in these MDPV was considered purely an incidental finding. The manner of death and cause of death are further illustrated in Fig. 10, which shows that the majority (92.1%) of the MDPV-positive individuals in the post-mortem material died of unnatural causes (V).

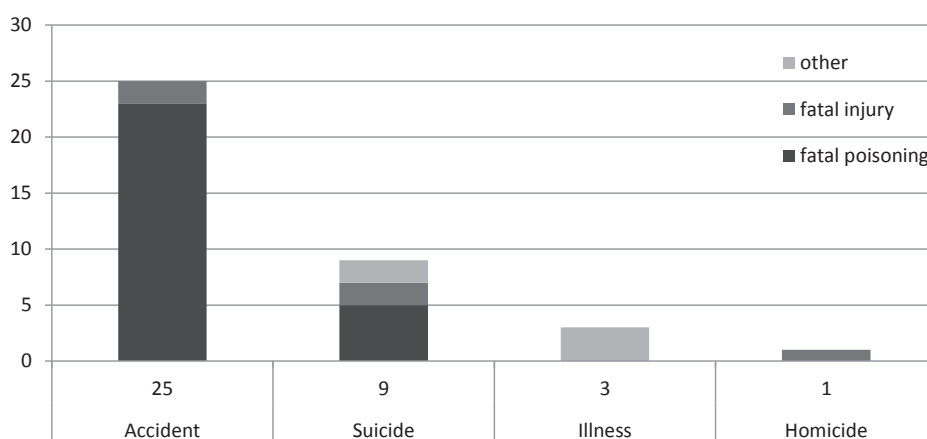


Figure 10. Frequency of different causes of death in the four death classes for deceased positive for MDPV in the study period in Finland (2009–2012) (V).

Suicide was more prevalent among those post-mortem cases positive for MDPV than among fatal poisonings of users of illicit drugs in general (24% vs. 6%) [11]. MDPV-positive suicide victims were also significantly younger than those MDPV-positive individuals who died of other causes (28.3 vs. 35.3 years, $p=0.039$, t-test). Previous research on suicide victims has revealed that drug users who have attempted suicide are indeed younger than drug users who have never attempted suicide [200]. Since the vast majority of the deceased in the study material had several other psychoactive substances besides MDPV in their system, it is impossible to estimate the role of MDPV in these cases. In only one of the suicides, was the victim known to have taken MDPV (among other substances) in order to commit the act. In other studies, cathinones have been found to be highly prevalent in suicides [84], which may suggest that the substances possess properties that induce suicidal thoughts (V).

5.1.3 Clinical assessment of impairment in MDPV-positive cases

In the clinical assessment of driving impairment, some of the MDPV-positive drivers did not show any signs of drug induced impairment (I). However, even though in most cases the

overall psychomotor performance was found to be within the normal range, some functional impairment was detected in 84% of the MDPV-positive cases. The most prevalent aberrations detected in the examination included difficulty in defining the current time, walking on a straight line, turning around, and speech, which all are symptoms that have been observed in association with the use of synthetic cathinones [78]. In this study, however, it was not possible to determine to what degree these aberrations were due to MDPV since the drug was in most cases found together with a variety of other psychoactive drugs. Another problem in the study material was that the clinical assessment of impairment was not performed in every case, even though it is legally required in all cases of suspected DUID (I).

5.1.4 Police confiscations of MDPV

The analysis of confiscated MDPV samples revealed that MDPV and phenazepam were sometimes sold as a mixture. The Finnish Police confiscated 219 batches of MDPV in the one-year study period (between 28 August 2009 and the end of August 2010). At the time, MDPV accounted for 45% of all confiscations of NPS and was the most prevalent NPS among all confiscated substances. The confiscated MDPV samples were found to originate in China (I).

5.1.5 MDPV before and after it was banned

The incidence of MDPV both in DUID and in post-mortem investigations decreased after it was banned (V). In DUID cases the decrease in the mean monthly numbers was 51.1%. After banning, the numbers of DUID cases positive for MDPV stabilised at a level of 5 to 10 cases per month. In post-mortem investigations, MDPV-positive cases became quite sporadic after the banning (V).

The multi-drug use pattern in the MDPV-positive DUID cases changed to some extent after MDPV was banned. The percentages of drugs used together with MDPV before and after the banning are illustrated in Fig. 11.

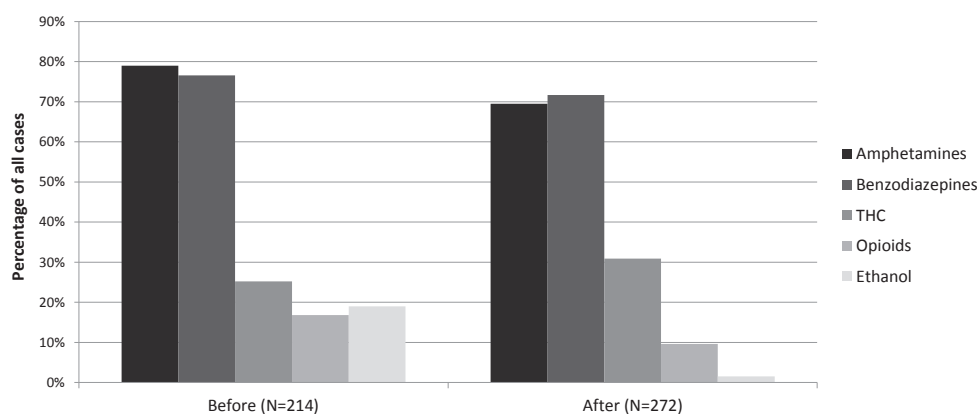


Figure 11. Other findings in MDPV-positive drivers before and after the banning of MDPV (June 2010).

When looking at the toxicology findings of the DUID drivers in court cases before and after the banning of MDPV, a change was observed in that amphetamines and opioids were significantly more often found together with MDPV before it was banned than after it (Fig. 11) ($p < 0.02$, Fisher's exact test). This finding is, however, somewhat skewed due to the fact that before it was banned MDPV was not screened for in every sample but it was after banning. Assuming that the observed change in the concomitant use of other drugs after the banning was real and not just a reflection of differences in study populations, this could suggest a change of user population over time. The temporal evolution in the use of NPS seems to be from initial use by established drug abusers, who frequently inject amphetamine and/or opioids, then expanding into a larger population of experimenters who less frequently use amphetamines and/or opioids. This temporal evolution in the user population for NPS could explain the results of an Australian study in which a geographic shift of MDPV use was observed over time from high population density areas, where i.v. use of illicit drugs tends to be concentrated, towards detections of MDPV over a wider geographic area with lower population density, where i.v. drug users are less prevalent [81] (V).

5.1.6 MDPV in court cases

In order to see what had changed after MDPV was banned in terms of how the court discussed MDPV findings in the DUID cases that went to court, certain demographic characteristics of the defendants were investigated (V). The percentage of MDPV-positive defendants that were male rose from 76.7% to 93.2% after the banning. The mean number of charges (DUID and others) remained the same throughout the study period. Banning of MDPV had no effect on the percentage of MDPV-positive defendants without a fixed abode, which was high throughout the study period (around 37%) (V).

Before it was banned, MDPV was only mentioned in the court decisions of 4.7% of the MDPV-positive DUID cases whereas after the banning it was mentioned in 95.5% of MDPV-positive DUID cases. This is likely related to the legal system in Finland where, in cases in which the basis of the charge is the finding of substances categorized as "zero-tolerance drugs" (banned substances), the drugs must be identified in the documents reporting the court's decision. However, if the basis of the DUID court case is impairment, rather than the presence of a psychoactive substance (either banned or not banned), the court is not required to name the substance that caused the impairment but rather discuss the observations that demonstrate impairment. The high percentage of cases in which MDPV findings were mentioned in the court documents after it was banned are therefore not surprising since the finding of a zero tolerance substance is a stronger basis for prosecution than demonstrating driving impairment. The low percentage of cases in which MDPV findings were mentioned in the court documents before it was banned is disappointing given the effort that was made to provide the court with information in the statements of laboratory results that gave a realistic view of the probable negative impact of MDPV on driving performance. However, in the vast majority of the cases the court did not need the MDPV finding for the sentence since other already illegal drugs were present in addition to MDPV, enabling a direct conviction based on the zero-tolerance law (V). It is thus understandable that, before MDPV was banned, the court did not care to use the MDPV findings and perhaps complicate the prosecution.

5.1.7 Geographical distribution of MDPV

The results of this thesis are generally in good accordance with earlier studies in that the vast majority of all MDPV-positive DUID cases in the 4-year study period were from urban areas (Fig. 12) [VI,201]. However, while study VI found the majority of MDPV-positive DUID cases in the greater Helsinki urban area, two independent studies of community wastewaters carried out in 2012 in several cities around Finland, found that MDPV was most prevalent in cities in eastern Finland close to the Russian border [111,112]. A greater volume of traffic and DUI surveillance may explain the concentration of MDPV-positive DUID cases in Helsinki, and suggests that the distribution of DUID cases will not necessarily indicate the overall distribution of users since it does not include those who do not drive while intoxicated or do not get caught.

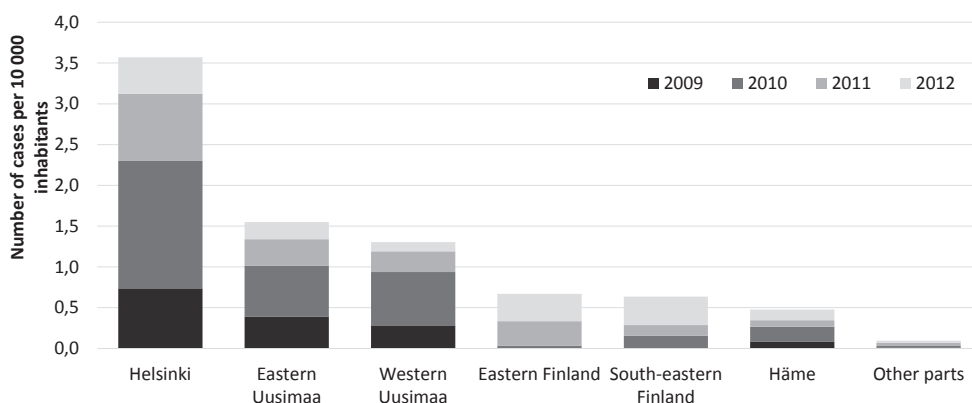


Figure 12. Geographical distribution of MDPV-positive cases in DUID investigations calculated as cases per 10 000 inhabitants [VI].

In this study the prevalence of MDPV was highest in the capital area during all years of the study, but in the year of the wastewater studies (2012), a major shift could be seen in case numbers from Helsinki towards eastern Finland (Fig. 12.) [VI]. The expanding geographical distribution can to some extent be due to the increased awareness and availability of the drug with time but that does not explain the very local popularity of MDPV at the eastern border. The more plausible explanation could be that the delivery routes to Finland may have been from Russia and that MDPV was thus readily available close to the border.

5.2 Phenazepam

In the one-year study period of the second part of this thesis, Phenazepam was screened in every sample from the apprehended drivers and was found in 141 (35%), which is a very high rate considering the illegality of the substance (II). Besides Finland, phenazepam has been frequently detected in the UK and Norway [117,202]. A considerable portion of phenazepam-positive drivers (29%) were apprehended in the South Karelia Police district which is an area in south-eastern Finland next to the Russian border (II). Phenazepam used in Finland is believed to originate mainly from Russia and the geographical distribution of the phenazepam-positive samples in this study give further evidence of that. Since the nationality of the driver is

not denoted in the background material received by the laboratory, some of the phenazepam-positive cases may be Russian drivers with a valid prescription for phenazepam. Unfortunately, no geographical distribution data from wastewaters in Finland is available for phenazepam since the two studies conducted recently did not include benzodiazepines [111,112].

The concentration of phenazepam in DUID cases was often high suggesting abuse of the drug. The median (range) phenazepam serum concentration in DUID cases was 0.061 mg/L (0.004–3.600 mg/L), which is lower than in a report on drivers from Georgia, US (0.17 mg/L) [89] but similar to a single case report from Houston, US 0.076 mg/L [88]. In nearly 50% of the cases in the study material, the concentration of phenazepam was above 0.070 mg/L which is the upper limit of the suggested therapeutic range in the treatment of anxiety [73]. The mean (range) age of all phenazepam-positive drivers was 33.1 (18–61) years and most of them (88%) were males (II).

In drivers, phenazepam was often found together with other benzodiazepines (43.3%) or amphetamine (67.4%) or with both (29.1%). The concentration of phenazepam was significantly higher in cases where there was no concomitant stimulant use (median 0.215 mg/L) when compared to the overall material. This is analogous to the MDPV findings where MDPV concentrations were higher in those cases in which no other stimulant was found. In only 7 cases was phenazepam the only psychoactive substance found in the blood of the driver (II).

In the course of this study, urine samples delivered together with the serum samples in some of the DUID cases (N=23) were analysed in addition to the serum samples. The active metabolite of phenazepam, 3-hydroxyphenazepam (OH-phenazepam) was the main substance in urine but the parent substance was also present in most cases, albeit in much lower concentrations (II).

In post-mortem investigations, phenazepam was screened in every sample and found in 17 autopsy cases in the study period. Phenazepam was in most cases found together with opioids (70.6%) and alcohol was also very often present (52.9%) (II). Benzodiazepines (and ethyl alcohol) combined with opioid use have been shown to markedly increase the risk of fatality by enhancing the respiratory depression caused by opioids [203].

In the studied post-mortem cases, phenazepam was never the primary cause of death, which is consistent with the general situation with other benzodiazepines. The median (range) phenazepam blood concentration in the post-mortem cases was 0.048 mg/L (0.007–1.600 mg/L) which is lower than in an earlier study of 20 Finnish post-mortem cases (0.09 mg/L) [204] (II). The median concentration in drivers was higher than in fatalities and also higher than the concentrations reported in fatalities in other studies [80,93]. Because of the low toxicity of phenazepam alone, other concomitant substances, typically intravenous opioids, are needed for a fatal outcome. The low concentrations found in phenazepam post-mortem cases may be an indication that it can potentiate the lethal effect of opioids even at lower concentrations.

In most phenazepam-positive cases in the post-mortem material, the cause of death was determined by the forensic pathologist to be accidental overdose with one or several psychoactive substances. Phenazepam was implicated in the death, together with other substances, in five cases out of the 10 fatal phenazepam-positive poisonings [VI]. All of the phenazepam-positive deceased were male and the mean age was 35 years. Just as in traffic cases, nearly two thirds of the fatalities with a positive phenazepam finding occurred in Eastern Finland close to the Russian border (II).

Phenazepam constituted 0.2% of the number of samples in police confiscations. The samples consisted mainly of tablets, capsules and powder, but also some fluids were found. Unlike the geographic distribution of phenazepam-positive traffic cases and fatalities described above, most

of the phenazepam was confiscated in Helsinki, while the South Karelia Police Department, close to Russian border, made up 23% of the confiscations (II).

5.3 Desoxypipradrol

Triggered by reports of cases of severe intoxication of young adults by an originally unknown designer drug, the screening of DUID samples for desoxypipradrol (2-DPMP) was initiated in 2010. In this study (III), DUID and post-mortem cases positive for 2-DPMP were investigated in a period of about 1.5 years between 2010 and 2012. In the study period, 106 (1.7%) of the confirmed DUID cases were positive for 2-DPMP. The monthly numbers were highest right after the start of the screening for the drug, which suggests that some drivers had assumed it would not yet be detected in the analysis of blood samples. In post-mortem investigations the percentage of cases positive for 2-DPMP was 0.05%; only 5 positive cases were detected in the study period. The median (range) 2-DPMP serum concentration in DUI cases was 0.065 (0.006–0.480) mg/L and in post-mortem blood 0.72 (0.01–1.4) mg/L (III).

Again, most of the drivers positive for 2-DPMP were males (85.5%). The mean age of 2-DPMP-positive drivers was 34.1 years (III).

Both in DUID and post-mortem cases, 2-DPMP was always detected together with various other psychoactive substances. In DUI cases the most prevalent co-findings were other stimulants (87.9%) or benzodiazepines (82.1%) or both (62.3%). Alcohol was present only in 2.8% (N=3) of the cases. In 19.8% of the DUID cases positive for 2-DPMP, the driver had also taken MDPV (III).

In all of the post-mortem cases, 2-DPMP was detected with other psychoactive substances. Opioids were present in most cases but alcohol in none. 2-DPMP was estimated by the forensic pathologist to have contributed to the death in two of the five 2-DPMP-positive post-mortem cases. In both of these, opioids were also present. The post-mortem blood concentration in both of these cases was 1.4 mg/L which is more than 20 times higher than the median 2-DPMP serum concentration in DUID cases (III).

Additionally, the results of the clinical examinations performed by a physician shortly after the arrest of the suspected driver were examined. The clinical examination for driving impairment was conducted in 57 of the 106 cases positive for 2-DPMP. Of these 57 examinations, psychomotor aberrations were observed in 69.7%. The most frequent aberrations included mood alterations, delayed/reduced pupil reaction to light, behavioural changes, problems in walking on a straight line and speech. However, on the three-step scale available for the physician for the assessment of the overall impact of these aberrations on the psychomotor performance of the suspect, only 39.4% of the drivers examined were judged as having been impaired while examined (III).

In a previous study of apprehended drivers in Germany, one of the subjects was found to be positive for 2-DPMP with a concentration of 0.36 mg/L which is at the upper end of the concentration range described in this thesis (III). The driver was assessed as showing signs of being under the influence of drugs by an examining physician and claimed to have had an episode of micro-sleep resulting in the car straying from the road and turning over. In addition to 2-DPMP, she had also a low concentration of MPDV and traces of benzedrone in her blood, but the likely cause of the observed impairment was judged to be 2-DPMP [78].

5.4 Pregabalin

Pregabalin was studied in DUID cases in 2012 over a period of one year (IV) in which a total of 459 samples (12% of all samples) were screened for the drug. There were 206 positive cases with median (range) serum concentration of 6.2 (0.68–111.6) mg/L which is in the upper third of the suggested therapeutic range (2–8 mg/L). The majority of the samples were above the upper limit of the typical therapeutic range. The highest concentrations in this study were well beyond the levels seen in fatalities [205, Table 2]. In DUID cases, pregabalin was unfortunately not analysed from every sample at the time of the study, but only when requested by the police or when the background information suggested use of pregabalin. Thus no assessments can be made of the prevalence of pregabalin from these numbers (IV).

Similarly to the other studied substances, in pregabalin-positive cases the concomitant use of other psychoactive drugs was extremely common. The mean number of other drugs in pregabalin-positive cases was 4.3, the highest number being 9. Some individuals may use several different drugs simultaneously under medical supervision in an appropriate manner. However, many of the DUI suspects in the study material showed concomitant use of pregabalin with illegal drugs, or pregabalin and a combination of other medicinal drugs that generally should not be used together, such as several different benzodiazepines or opioids. Of the pregabalin-positive drivers, 54.4% also had cannabis and 44.2% had amphetamine in their blood (IV). These findings are in good accordance with previous research on pregabalin in Finnish post-mortem samples where 48% of the pregabalin-positive cases were considered to be drug abuse rather than appropriate medical use [98].

The percentage of women in the DUID cases positive for pregabalin (20%) was higher than the percentage of women positive for the other drugs detected in apprehended drivers in this thesis. This could be because some portion of the pregabalin findings in women was due to appropriate medical use since, in earlier studies, consumption rates of prescription drugs in general have been shown to be higher for women than for men whereas the frequency of illicit use of prescription drugs is equal for both genders [206]. In this study, the percentage of female drivers that had, in addition to pregabalin, either amphetamines or cannabis in their blood was almost equal to that of males (29.3% vs. 29.7%) (IV).

Of the 206 cases positive for pregabalin in the study period, in three cases pregabalin was the only psycho-active drug detected. The concentrations in these cases were 17.9 mg/L, 18.2 mg/L and 0.7 mg/L. In one of the cases with a high concentration (17.9 mg/L) a clinical examination of impairment was performed and aberrations were found in walking on a straight line and pupil reaction to light. However, the overall psychomotor performance was considered by the examining physician to be within the normal range (IV). The only of previous research on pregabalin and driving impairment involved driving simulator studies with low drug doses [136,137]. Based on pharmacokinetic data for pregabalin, the driving simulator study which used the higher dose (150 mg t.i.d.) would result in an average blood concentration of 1.27 mg/L (range: 0.29–2.84 mg/L) which is at the lower end of the typical therapeutic range [171]. Therefore the information from these driving simulator studies is of limited use when considering possible impairment in the cases with very high blood concentrations described in this thesis.

Some users have reported feelings of euphoria with pregabalin [134,135,207], which may explain the otherwise inexplicable abuse of this drug. The results of this study provide further evidence that many users of illegal drugs also abuse pregabalin.

5.5 NPS in post-mortem cases

New substances were added to the toxicological screening program for post-mortem cases whenever there was reason to believe that a certain substance could be being abused in Finland and if there was a reference standard available for the substance. For some substances, confiscated material from Customs or the Police was used as reference material until commercial reference standards became available. NPS findings in post-mortem cases from 2009 to 2012 are illustrated in Fig. 13. The total number of findings in this time period was 95 in 75 separate cases [VI]. Phenazepam and pregabalin are not included in these numbers. Unfortunately, the dates when they were first included in the screening program are no longer available for all substances in Fig. 13., but they were included as soon as there was any indication of their abuse in Finland. It is thus likely that very few cases positive for these NPS were not detected in the study period.

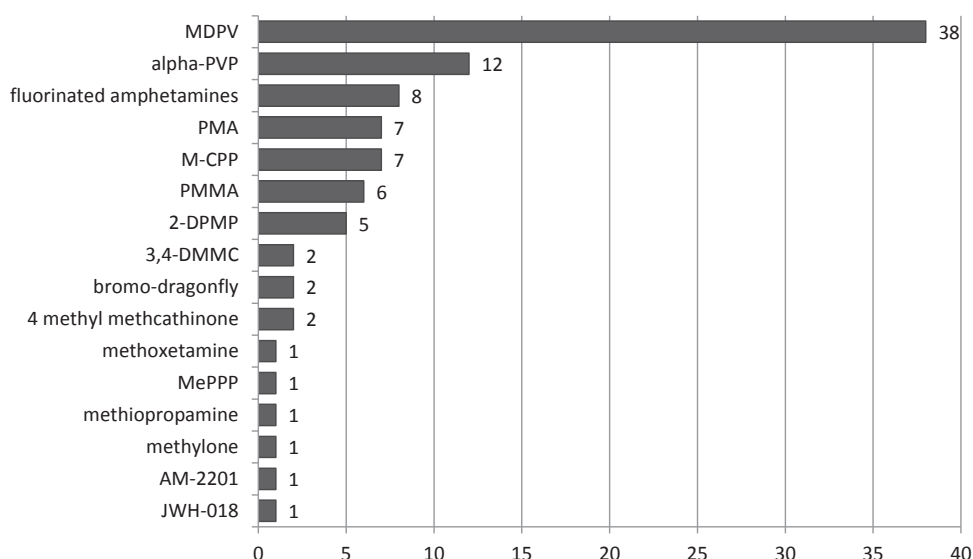


Figure 13. Number of NPS findings in post-mortem investigations in 2009-2012 (excl. phenazepam and pregabalin) [VI].

As can be seen from Fig. 13., synthetic cannabinoids are almost completely absent from the post-mortem statistics in Finland even though they have been included in the analytical methods since 2009. JWH-018 and AM-2201 were both from one case which was also positive for 4'-methyl- α -pyrrolidinopropiophenone (MePPP) [VI]. In other countries synthetic cannabinoids have been occasionally detected in post-mortem samples [208,209,210,211]. The EMCDDA annual report on NPS lists synthetic cannabinoids as the single biggest group of emerging substances in the last 3 years and the group has grown every year more than other drug groups [9]. Despite this, however, they have not appeared more frequently in the post-mortem statistics in Finland suggesting either lower toxicity of the substances when compared to that of other NPS, e.g. synthetic cathinones, or, that the prevalence of the use of synthetic cannabinoids is lower in Finland than in other countries. In drug-related death cases in Finland, the main intoxicant has generally been an opioid – often used together with alcohol or other CNS sedatives.

Of the deceased positive for any of the NPS in the study period, 78.7% were males. Mean (range) age was 33 (17–63) years. For most, the manner of death was determined by the forensic pathologist to be accident (60.0%) (including accidental overdose), which is in accordance with previous research on users of illicit drugs [212,213]. The frequency of other manners and causes of death, using the categories defined by Finnish forensic pathologists, are illustrated in Fig. 14. The cause of death was in most cases (64.0%) fatal poisoning by illegal or medicinal drugs and in most of these cases (58.3%) one or several NPS were mentioned as having directly contributed to the death. Of these 75 deaths, 88.0% were assessed to be deaths by unnatural causes [VI].

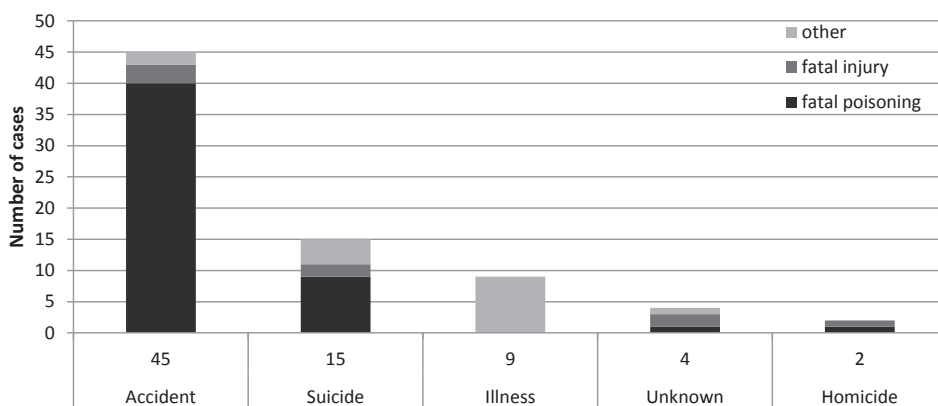


Figure 14. Frequency of different causes of death (fatal poisoning, fatal injury, other) for each of the five manners of death for deceased positive for any NPS in the study period in Finland (2009–2012) (phenazepam and pregabalin excluded) [VI].

There were three fatal traffic accidents in the study period in which NPS were present [VI]. Two of the victims had been driving motorcycles and one was the driver of a car. MDPV was present in two of the cases (0.07 and 0.059 mg/L), in the latter case together with alpha-PVP (0.6 mg/L). The third driver was positive for 3,4-DMMC (0.06 mg/L) [VI].

6 GENERAL DISCUSSION

Driving under the influence of alcohol and drugs presents a serious risk to everybody on the road. According to EMCDDA, alcohol is involved in about 25% of the traffic accidents in Europe but the role of drugs in traffic accidents is harder to estimate due to the limited amount of data available [214]. NPS constitute a new and worrisome threat to traffic safety although the magnitude of that threat is poorly understood since information on their prevalence and impact on driving ability is limited. In this thesis, new information on NPS was obtained from DUID and post-mortem investigations and from DUID court cases.

The toxicology results from DUID investigations proved to be excellent material for studying prevalence and user profiles of NPS since all of the studied substances were frequently detected and thus a large body of results was available for examination. Post-mortem investigations also provided data, albeit with a substantially lower number of cases. Police confiscations, results from clinical impairment tests on apprehended drivers and court documents on DUID cases also contributed to the data.

One of the main goals of this study was to obtain additional information on prevalence and blood concentrations of NPS to supplement the very limited amount already available. Before the initiation of the study, the appearance of MDPV in Finland in particular had been widely reported in the news and had been the subject of much discussion. Despite this public interest, the true prevalence of NPS use was unknown. Among the findings of the study, the high prevalence of 2-DPMP in Finland was especially unexpected since the international prevalence of 2-DPMP was – and still is – quite limited. Phenazepam, on the other hand, had been present in Finland for a long time but at the time of the study it was not yet banned. The magnitude of the impact of phenazepam use on driving safety in Finland may have been underestimated earlier since the extent of its use was not generally known. In the case of pregabalin, a number of reports on its abuse potential had been published by the time this study was initiated [97,127,128,215,216] but little information was available on the effects of pregabalin on driving performance [136,137] or on the concentrations associated with abuse of the drug (Table 2). This situation prompted its inclusion in the examination of findings in DUID suspects. The DUID and post-mortem cases studied in this thesis proved to be an outstanding material with which to expand understanding of the toxicology profile of the selected NPS at the time when the problems their abuse creates were being increasingly recognised.

6.1 Analytical aspects and methodological considerations

The problems and questions faced in the toxicological analyses of DUID investigations all over the world are for the most part alike. The lack of scientific information on NPS and lack of commercially available reference standards greatly complicate screening NPS in human samples. The International Council on Alcohol, Drugs and Traffic Safety (ICADTS) guidelines for research on DUID recommends that every driver be screened for the same panel of drugs. The analytical approach applied in the individual DUID studies in this thesis was not completely in accordance with this guideline since it was not designed for research purposes but rather for routine investigation of more than ten thousand cases every year. In order to meet the needs of the police and best support any prosecutions arising from the cases, the range of drugs screened was largely determined by the nature of the preliminary charges filed by the police [217]. The

practice adopted was a compromise between ensuring an efficient process while including a reasonable spectrum of abused, illicit and licit drugs.

The analysis of DUID and post-mortem samples was performed in two separate laboratories utilising somewhat different analytical approaches. Both laboratories had, however, acknowledged the pitfalls of immunological screening of drugs in forensic toxicology and proceeded to comprehensive target screening with MS-based methods. For the analysis of post-mortem samples, and especially in cases of fatal intoxication where every finding may be relevant, the LC-TOFMS screening has proved very useful. Of the NPS-positive post-mortem cases in the study period, one or several NPS were implicated to the death in more than half of the cases [VI].

The study periods in the individual studies included in this thesis were in most cases selected in such a manner that the substance in question was analysed from every sample in the study period. This was true for all post-mortem investigations. In the DUID investigations there were, however, two exceptions. (1) At the beginning of the first study period (2009), MDPV was not analysed from every sample but only in cases where there were amphetamines present in the sample or there was otherwise reason to believe that the person had taken MDPV (I). It is likely that few (if any) MDPV-positive samples were not analysed since later, when the process was changed so that every sample was screened for MDPV (2010), the majority of MDPV-positive samples also had amphetamines present (I,V). (2) For the whole study period of the pregabalin study (2012), pregabalin was only analysed when requested by the Police. At the time, pregabalin was studied from 12% of all samples. It is likely that many pregabalin-positive cases were missed and thus no estimates on the prevalence of the drug could be made.

It is reasonable to assume that not all individuals driving under the influence of drugs in Finland are apprehended. Suspected DUI drivers are in many cases apprehended due to fellow citizens' reports on unsafe driving or observed use of intoxicants before driving. Driving errors observed by the Police are also common reasons behind the apprehension. It is, however, not particularly common to catch a drugged driver in random stop checks. Thus it is likely that at least those drivers that are seriously impaired by the use of drugs will be caught in this system. Drug users that do not have access to a motor vehicle or are so intoxicated that they cannot even enter a car are not included in the DUID material used in this thesis.

In forensic post-mortem investigations in Finland, all unexpected deaths are autopsied and in most cases also examined toxicologically. It is likely, that very close to 100% of all drug related deaths undergo a thorough post-mortem toxicological analysis in Finland. Thus the coverage of the post-mortem material is excellent for research on drug prevalence and toxicity.

6.2 Clinical impairment testing in DUID-cases

Many policemen are extremely skilled in detecting drug use in a driver, as indicated by the fact that over 90% of the samples sent to laboratory analysis are positive for at least one psychoactive substance (police statistics from 2012). The roadside screening test mostly used in Finland (DrugWipe®) has been shown to perform well in cases of amphetamine use but to a somewhat lesser degree with e.g. opiates or cocaine [141,218]. The clinical tests performed after the arrest in many of the studied cases only provided suggestive evidence of impairment. Poly-drug use in the studied cases also complicated the interpretation of the test results. The low frequency of detection of driving impairment in the clinical tests, despite the fact that most subjects were apprehended because they had been observed to be driving improperly, is of great concern and has been reported by other investigators and the writer has additional experience of the

problem. For example, a clinical examination was performed on one driver from the non-NPS use population and the physician judged him as not impaired. However, his blood results later revealed that he had 12 mg/L temazepam in his blood (among other substances) – a concentration that is associated with severe sedation and would surely have affected driving, even if tolerance had occurred [VI].

Another troublesome issue is whether tolerance should be taken into account in those DUID cases in which medicinal drugs are involved. Patients appropriately taking prescribed medications for long periods may develop tolerance to some of the pharmacological effects that impair driving. It could be argued that such individuals may not be impaired at blood levels that would impair others and therefore should not be penalised. However, the drivers in the study material had initially been apprehended for some concrete reason, accident, unsecure driving or similar, thus it is likely that the drug they had taken had indeed caused driving impairment and affected their driving behaviour. Furthermore, alcohol too induces tolerance but still the legal limits are the same for everybody, from moderate drinkers to alcoholics – why should the rules be different for other substances that affect the CNS?

6.3 Concomitant use of other drugs in cases positive for NPS

A characteristic phenomenon in all subsections of this study was that in the vast majority of the DUID and post-mortem cases the subjects positive for any of the studied substances were poly-drug users. This is in accordance with previous studies on users of illegal drugs in Finland and other Nordic countries [11,140,219,220,221]. The studied NPS were nearly always used together with a spectrum of other psychoactive drugs from different pharmacological groups. Other studies have shown that users of NPS have on average more previous experience from the use of illegal drugs than individuals that do not report use of NPS [222,51]. The results of the present study suggest that single experimenters and occasional users either do not get caught for DUI by the Finnish system or there are few such individuals in Finland. It could be that occasional experimenters are still relatively well in control of their lives and thus try to avoid colliding with generally accepted norms such as “driving under the influence of drugs is dangerous and thus forbidden”. When drug use advances into dependence/addiction, either such good intentions no longer exist, or, the loss of control over behaviour means that the good intentions cannot be put into practice. In some cases, especially with drivers suffering from the antisocial personality disorder commonly encountered in problem drug users, colliding with the prevailing norms is one of the goals of driving under the influence [223]. Due to these factors, the common means of education about the dangers of DUID, or drug abuse in general, may have limited ability to regulate the behaviour of problem drug users.

6.4 User categorisation and differences between the studied substances

Even though users of NPS in this study share many factors and qualities regardless of the substance used, some differences between the studied substances could be observed in the concomitant use of other drugs. The drivers could be categorised into two groups according to the primary drug they are using: stimulant users and sedative users. This phenomenon was especially prominent in phenazepam-positive cases. The first subgroup of phenazepam users had taken phenazepam as a

part of a spectrum of sedatives with relatively high concentration of the drug whereas the other group had used smaller doses of phenazepam presumably in order to manage the side effects of stimulant use. Alcohol was seldom used together with the studied stimulants, MDPV and 2-DPMP, but more often encountered with the two CNS sedatives, phenazepam and pregabalin. The same was true for opioids in that they too were rarely used with the stimulants but more often with the sedatives. In post-mortem cases, however, stimulant NPS were frequently present together with opioids, suggesting that these stimulants are not particularly toxic on their own but may contribute to the fatal outcome when used together with opioids. In previous studies, opiate users have been shown to possess a higher mortality risk than those using amphetamines [212,224]. Also in this study, opioids were proportionally much more often present in post-mortem cases than in DUID cases suggesting elevated mortality of NPS users who also abuse opioids. Apprehended drivers using stimulant NPS had high concentrations of the stimulant often combined with a modest concentration of sedatives, in most cases benzodiazepines. On the other hand, drivers with high concentrations of sedatives in most cases only had little or no stimulants in their blood.

Previous research has suggested that the NPS users are younger than users of more established abused drugs [225]. This was, however, not confirmed in this thesis since the drivers in the study material were slightly older than in all DUID cases in Finland in 2007 (34.0 vs. 32.1 years) and the MDPV-positive deceased were about the same age as the mean age in all fatal poisonings of users of illicit drugs in Finland in 2012 [11].

Both in DUID and post-mortem investigations, MDPV-positive females were significantly younger than MDPV-positive men. Similar observations on gender differences in the age profile of NPS users have not been reported elsewhere and the reason for these considerable age differences remains, for the most part, unclear. These age differences are in conflict with previous results on gender differences in drug use in general, although the earlier research has mostly concentrated on age at first drug use [226]. The finding is also in conflict with previous studies on Finnish DUID offenders where no age differences were found between genders [140]. There are studies that have revealed differences between the two genders in terms of the acquisition, escalation and relapse phases of drugs use [227] and it is likely that these differences are also present in the usage of NPS. Some studies have suggested that females are more likely to abuse stimulants than other classes of drugs [227,228]. In this thesis, however, as is true for most studies on drugs users, the number of males was about three times that of females for every studied substance.

6.5 Court cases of MDPV-positive apprehended drivers

As a part of the study, a group of drivers positive for MDPV was characterised more closely by looking at the court documents of the DUID cases. It was found that many of the MDPV-positive drivers were well outside mainstream society since more than a third of them were without a fixed abode and many were very frequent visitors at the district court. Similar results for DUID offenders have been seen in a previous Finnish study [229]. Based on the results from this thesis, it is suggested that the high proportion of individuals without a fixed abode can be generally attributed to the social status of users of MDPV – it does not seem to be a trendy club drug for the affluent but rather is used by individuals with a history of drug use and little control over their choices in life.

6.6 Geographical distribution on NPS and changes in prevalence

In terms of geographical distribution, MDPV was mainly detected in the capital area whereas a considerable portion of the phenazepam cases were from the area close to the Russian border. This probably reflects the delivery routes of the two drugs but also differences in drug use patterns in urban and rural areas. NPS have in general been found to be more prevalent in high population density locations, colleges and universities [201]. The geographical distribution of MDPV changed somewhat over time probably indicating the development of new distribution channels or users' awareness of the existence of the drug. However, at the same time the number of MDPV-positive cases decreased after it was banned in Finland. In addition to the impact of banning MDPV, the observed decrease both in DUID and post-mortem cases positive for MDPV could also be due to the typical "evolution" or "life cycle" of NPS [230]. Earlier studies from the UK have suggested that changes in prevalence may reflect alterations in price, quality and availability of the drug rather than being a direct consequence of the banning [222]; this may be true in Finland as well.

The length and character of the life cycle seems to vary between the studied NPS. MDPV was common for several years and in 2015 it is still encountered both in DUID and post-mortem cases, albeit much less often than during the peak years. The other studied stimulant, desoxypipradrol, never became an internationally popular stimulant the way MDPV did. It was mainly encountered in Finland and the UK and only for a limited time. Desoxypipradrol is still occasionally found in the post-mortem investigations in Finland but while MDPV clearly took its place among the more established drugs of abuse in Finland, desoxypipradrol findings have become quite rare after the brief peak in 2010–2011. Currently in 2015, α -PVP is the most common stimulant NPS in Finland.

At present, all new substances are equally available all over the world, thanks to the Internet, which lowers the threshold of experimenting with new substances. The fact that Finland has been among the most active countries in reporting new substances to EMCDDA suggests that even though Finland is not located on the main delivery routes of abused drugs, Finns enjoy trying new substances perhaps even more eagerly than other Europeans. However, the comparison between countries is somewhat difficult since not every country has the same possibilities and prerequisites for continuously adding new substances into the screening programs.

6.7 MDPV in suicides

In terms of the manner of death in the post-mortem cases studied in this thesis, MDPV was proportionally more prevalent in suicides than the more conventional abused drugs. Some earlier studies have suggested that the use of synthetic cathinones may be associated with depression and schizophrenia-like symptoms [56,231,232,233], which could partly explain the findings in this study. Unfortunately, the background information in the post-mortem cases frequently does not include sufficient history of mental illnesses of the deceased, which complicates any further interpretations on the issue. However, MDPV has gained a reputation as a "bad drug" with unpleasant come-down effects, which may explain some of the fatal outcomes in the study material [25].

6.8 Pregabalin as an example of prescription drug abuse

Pregabalin has been available as a medicinal drug for little over 10 years and has gradually gained popularity in the treatment of epilepsy and neuropathic pain, for which it has been approved, and also in some off-label indications such as migraine and insomnia [234,235]. In the present study, pregabalin was detected in high concentrations and mainly together with other abused drugs. Of all the 206 cases in which pregabalin was found, in only one of them could it have been used for an appropriate medical purpose. At the time of the study in this thesis, pregabalin was generally not prescribed for users of illegal drugs in Finland since clinicians had already noticed that addicts took large doses of pregabalin in order to boost the euphoric effects of opioids they were abusing [personal communication]. Therefore, finding pregabalin in combination with illegal drugs allowed the assumption that pregabalin was being abused.

In all of the individual studies on DUID cases in this thesis the studied substances were found in high concentrations. In post-mortem cases, however, the concentration of NPS was not always particularly high, indicating that the NPS did not play a significant role as a sole intoxicant in fatal poisonings. The number of cases studied is exceptionally high when compared to previous research on NPS. The reported cases cover both genders and a broad age range providing reliable reference material for the interpretation of forensic and clinical toxicology data.

7 CONCLUSIONS

When the analysis of blood samples for DUID cases is performed in a standardised and comprehensive manner so that most drugs of abuse are likely to be detected as soon as possible after they have entered the drug marketplace, the data obtained provide an excellent platform to study the prevalence and user characteristics of NPS. There are more findings of abused drugs in DUID cases than there are in post-mortem investigations so substances with low prevalence are more likely to be encountered amongst DUID cases. In this thesis, both sources of data have been utilised and together they provide an excellent body of material for research.

In the studies included in this thesis, serum concentrations of the NPS in drivers were often high, even when compared with the blood concentrations in fatal poisonings, suggesting significant impairment of psychomotor performance and driving ability. The incidence of MDPV decreased after it was banned both in DUID and in post-mortem investigations. Both in drivers and deceased, the NPS were usually found together with more established drugs of abuse. This was true also with pregabalin; in most of the pregabalin-positive cases drugs of abuse were also found indicating that pregabalin was being abused rather than being used for a prescribed medical purpose.

Drivers suspected of DUID could be categorised into two distinct groups: those whose drug use suggested that they were seeking a sedative “high” and those whose drug use suggested they were seeking a stimulant “high”. Significant differences in the blood concentrations and concomitant use of other drugs were found between these two groups. Even though sedatives were used by both groups, their serum concentrations were significantly higher in individuals who did not take stimulants concomitantly and it seems likely that the stimulant users took sedatives to manage stimulant side-effects.

The frequency of suicide was extraordinarily high in those post-mortem cases which were positive for MDPV. Since the overall number of MDPV-positive post-mortem cases was low, interpretation of this data is best left until additional information is available. However, similar results have been obtained in other studies on synthetic cathinones.

Since NPS are usually detected along with other more established, banned, drugs of abuse, the importance of NPS finding for the prosecution of DUID cases is limited. The drivers would have been prosecuted even without the detection of the NPS because of the presence of the banned drugs. However, NPS have adverse effects on the psychomotor performance of the driver and may interact in unpredictable ways with other psychoactive drugs (although there is limited data to indicate the magnitude of such effects), increasing the risk of accidents and serious or life-threatening injury for the driver and the public. Providing regulatory authorities with the information needed to make early decisions on banning NPS will help to minimise these risks.

In fatal poisonings positive for NPS, the forensic pathologist frequently implicated the NPS in the cause of death, usually along with other substances. Thus, for post-mortem investigations, it is important to include new substances into the screening program as soon as possible after they appear in the user population in order to provide the forensic pathologist with all the relevant toxicological data about the case.

After the termination of the study described in this thesis, new substances (e.g. α -PVP, PV8, ethylphenidate, etizolam) have emerged in addition to further cases involving the NPS studied in this thesis. Monitoring NPS will continue and, where possible, also the assessment of trends in the use of NPS. The rapid emergence of new substances creates an endless demand for research

on NPS in order to provide health care professionals, toxicologist, clinicians and law-makers with necessary information. The abuse of prescription drugs is a growing phenomenon which also needs to be further investigated so that the correct dimensions of the problem and suitable measures to handle it can be developed.

Future research is needed in order to understand the epidemiology, pharmacology, clinical effects, and management of the problem of new abused drugs. Efficient and reliable analytical detection of these substances are required to enable their monitoring in different situations such as those studied in this thesis (DUID and post-mortem cases) and others such as drug rehabilitation institutions, drop-in centres, needle exchange programs, youth drug recovery homes, etc. The results presented in this thesis, and those of future scientific research, will help to increase the body of knowledge on NPS and to contribute to reducing the harm associated with their abuse.

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